# MECHANISMS OF SELECTIVE INDUCTION OF GASTRIC MUCOSAL EICOSANOIDS IN RESPONSE TO POTENTIALLY NOXIOUS STIMULI

1991

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## ABSTRACT

Title of Dissertation: Mechanisms of Selective Induction of Gastric Mucosal Eicosanoids in Response to Potentially Noxious Luminal Stimuli

Elizabeth Ann Montcalm-Mazzilli, 1991

Dissertation directed by: Terez Shea-Donohue, Ph.D., Research Assistant Professor of Medicine and Physiology

Gastric mucosal eicosanoids, such as prostaglandins and leukotrienes, are released in response to injurious agents. Prostaglandins however, have a protective action on the mucosa while leukotrienes have been associated with the production of mucosal damage. Although prostaglandins and leukotrienes are both derived from arachidonic acid, they may be elaborated by different cell types. Hypothesis: exposure of the gastric mucosa to potentially noxious but physiological stimuli involves a selective induction of leukotrienes and prostaglandins. Mucosal leukotriene $C_4$ ($LTC_4$) and prostaglandin $E_2$ ($PGE_2$) generation was evaluated following intragastric administration of an 80 ml load of water (control), acid (50 or 100 mM HCl) or bile salts (5mM). One hour later, mucosal biopsies from the antrum and the fundus were obtained by gastroscopy for histological evaluation and for the ex vivo determination mucosal $LTC_4$ and $PGE_2$. There were no differences in histological scores among treatments. $LTC_4$ generation (pg/mg/protein) was undetectable after water ($1.69\pm1.60$ antrum and $0.01\pm0.0$ fundus) but was significantly ($p<0.05$) stimulated after 50 mM acid ($14.23\pm1.24$ antrum and $13.04\pm2.77$

fundus), 100 mM acid (13.27±1.67 antrum and 9.62±2.99 fundus) or bile (12.85±1.20 antrum and 12.27±2.80 fundus). In contrast, there was a measurable release of $PGE_2$ (ng/mg/protein) after the water load (55.06±6.97 antrum and 40.99±8.59 fundus) which was significantly increased by bile (88.29±10.66 antrum and 72.30±9.39 fundus) but not by acid. These results suggest that prostaglandins and leukotrienes are released from different cell types which can be selectively induced. A neural mechanism was investigated because of the close association between nerves and mast cells, the proposed source of leukotrienes. Lidocaine (2.2 mg/kg iv bolus followed by 66 μg/kg/min iv infusion) was used to inhibit sensory afferents. Lidocaine significantly inhibited $LTC_4$ generation following acid or bile, but had no effect on $PGE_2$ synthesis after bile. Thus, the release of $LTC_4$, but not $PGE_2$, in response to luminal stimuli may be neurally mediated.

# MECHANISM OF SELECTIVE INDUCTION OF GASTRIC MUCOSAL EICOSANOIDS IN RESPONSE TO POTENTIALLY NOXIOUS LUMINAL STIMULI

by

Elizabeth Ann Montcalm-Mazzilli

Dissertation submitted to the Faculty
of the Department of Physiology
Graduate Program of the Uniformed Services University of the
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requirements for the degree of
Doctor of Philosophy 1991

## DEDICATION

For Daddy.

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Something of this magnitude can not be accomplished alone. I would like to thank the members of my advisory committee, Drs. Jack E. MacKenzie, Gregory P. Mueller, Donald Sellitti and James M. Terris for their support and guidance over the years it took to accomplish this doctoral program. Dr. Sellitti was especially helpful dealing with problems with my histology data. It is also necessary to thank the members of the Digestive Diseases Division, Department of Medicine. Dr. Andre Dubois was the person who initially hired me to work at U.S.U.H.S. and all of the laboratory work was done in this division's laboratory. Many of the people that were in the Department of Medicine when I started school have left, however, many of these people are still my friends and they are still very supportive. Our lunch crowd has expanded from just two people to ten people. We have seen each other through many good times and a few sad times.

I also have to acknowledge the support of my family, my Mom who always told me that I could do it and my Dad, who didn't get to see me finish and receive my degree. My husband, Michael, deserves a special mention. Although he did not help me type, he was always willing to argue with me about what my data meant. I would also like to thank Michael's parents; they helped me with computer problems at all hours of the day and night.

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| | |
|---|---|
| AA | arachidonic acid |
| ANS | autonomic nervous system |
| ASA | aspirin |
| CGRP | calcitonin gene related peptide |
| CM | circular muscle |
| CO | cyclo-oxygenase enzyme |
| CONCN | concentration |
| DRG | dorsal root ganglion |
| FER | fractional emptying rate |
| 5-LO | 5-lipoxygenase enzyme |
| HETE | hydroxy-eicosatetraenoic acid |
| HPETE | hydroperoxy-eicosatetraenoic acid |
| IG | intragastric |
| INDO | indomethacin |
| IV | intravenous |
| LM | longitudinal muscle |
| LT | leukotriene |
| MMC | mucosal mast cell |
| MP | myenteric plexus |
| ND | nodose ganglion |
| NDGA | nordihydroguaiaretic acid |
| NSAID | non-steroidal anti-inflammatory drug |
| NTS | nucleus of the solitary tract |
| PG | prostaglandin |
| SEC | surface epithelial cell |
| SEM | standard error of the mean |
| SC | subcutaneous |
| SP | substance P |
| WY | WY48,252 |

# BACKGROUND

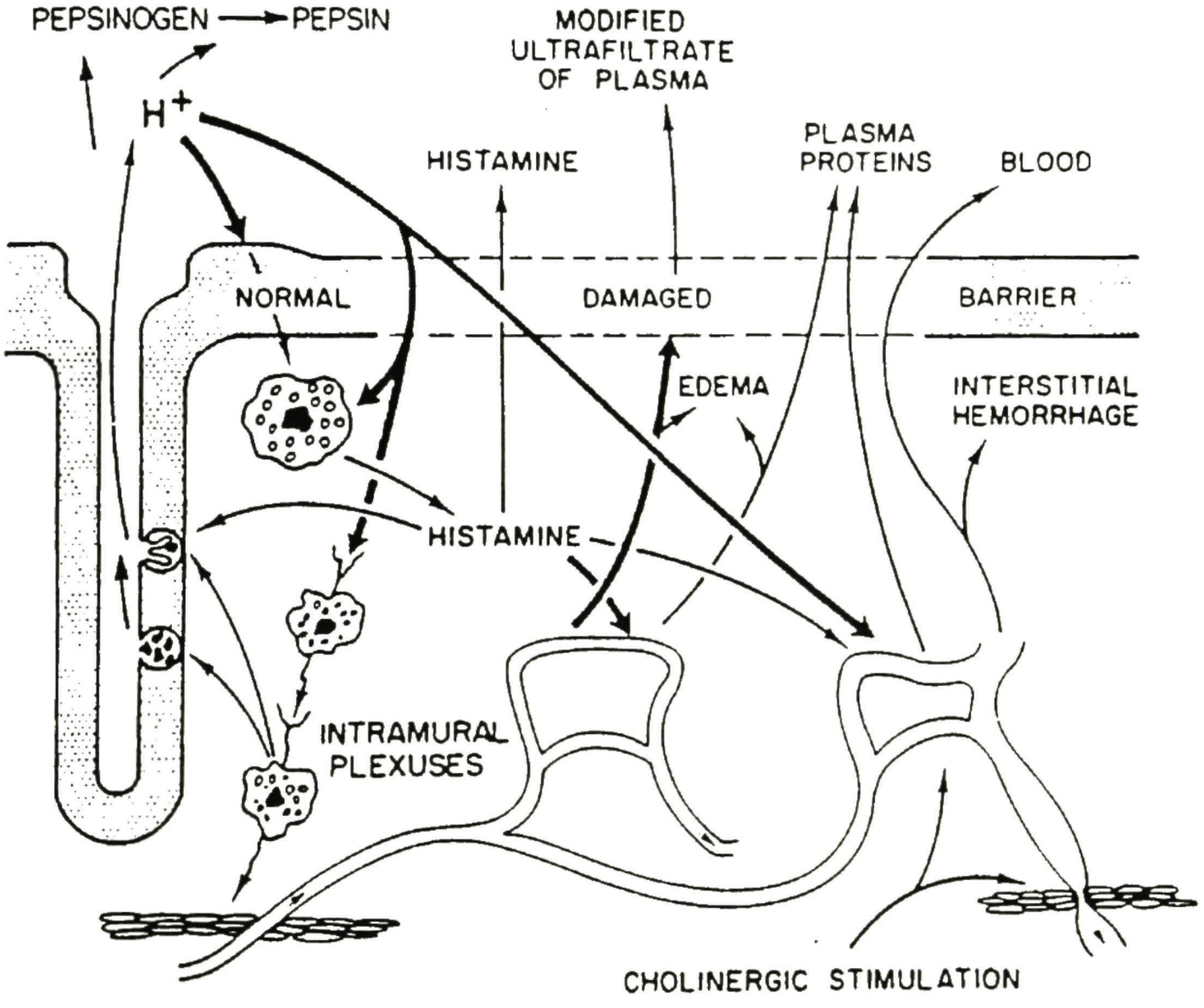
## The Gastric Mucosal Barrier

The concept of the gastric mucosal barrier was proposed by Davenport in 1964. Originally, the term referred to the passive permeability characteristics of the mucosa. However, recent investigations have begun to illustrate the complexity of the gastric mucosal barrier. Studies dealing with the "disruption of the barrier" have equated this disruption with an increase in mucosal flux of hydrogen, sodium and potassium ions. When the gastric mucosal barrier is broken, the high concentration gradient of hydrogen ions across the gastric mucosa causes hydrogen ion to enter the mucosal cells in large amounts (back diffusion). Ultimately, the response to unchecked acid back diffusion is vasodilation, edema, surface epithelial cell (SEC) necrosis and sloughing and mucosal hemorrhage (Figure 1).

The gastric epithelium possesses a remarkable ability to resist autodigestion from the high intraluminal acid concentrations to which it is continually exposed. During periods of peak acid output, acid concentration can reach 150 mM which corresponds to a pH of approximately 2.0 (Granger, 1988). This pH is capable of denaturing protein. While the precise mechanisms responsible for the resistance to acid damage remain to be elucidated, the ability of the gastric epithelium to resist injury is truly impressive.

There exists a group of agents called "barrier breakers" that disrupt the structure of the gastric mucosal barrier. Endogenous barrier

Figure 1. Effect of excessive "back diffusion" of acid on the gastric mucosa. From Davenport, 1977.


PEPSINOGEN → PEPSIN
MODIFIED ULTRAFILTRATE OF PLASMA
H+
HISTAMINE
PLASMA PROTEINS
BLOOD
NORMAL
DAMAGED
BARRIER
EDEMA
INTERSTITIAL HEMORRHAGE
HISTAMINE
INTRAMURAL PLEXUSES
CHOLINERGIC STIMULATION

breakers include bile salts, lysolecithin and pancreaticenzymes. Bile salts are particularly important barrier breakers since reflux of bile from the duodenum into the stomach has been shown to cause gastric erosions. Bile salts are thought to change the permeability of the gastric mucosa via their detergent action. Conjugated bile salts, having a pKa of about 1.8, cause a greater degree of damage in the presence of a low pH. In contrast, deconjugated bile salts have a higher pKa (about 5) and thus are less damaging at a low pH. Silen et al. (1975) have shown that the deconjugated bile salts are approximately 4 times as potent on a molar basis in damaging the gastric mucosa when compared to the conjugated bile salts. The concentration of bile salts found in the gastric juice of man with gastric ulcer has been reported to be as high as 7.4 mM (Black et al., 1971). Besides disrupting the gastric mucosal barrier, bile salts have been shown to allow increased back diffusion of hydrogen ions into the gastric mucosa of dogs (Black et al., 1971) and man (Ivey et al., 1970). Thus, the gastric mucosa may be exposed to hydrogen ion and bile salts under physiological conditions and these agents have the potential to produce damage to the gastric mucosa.

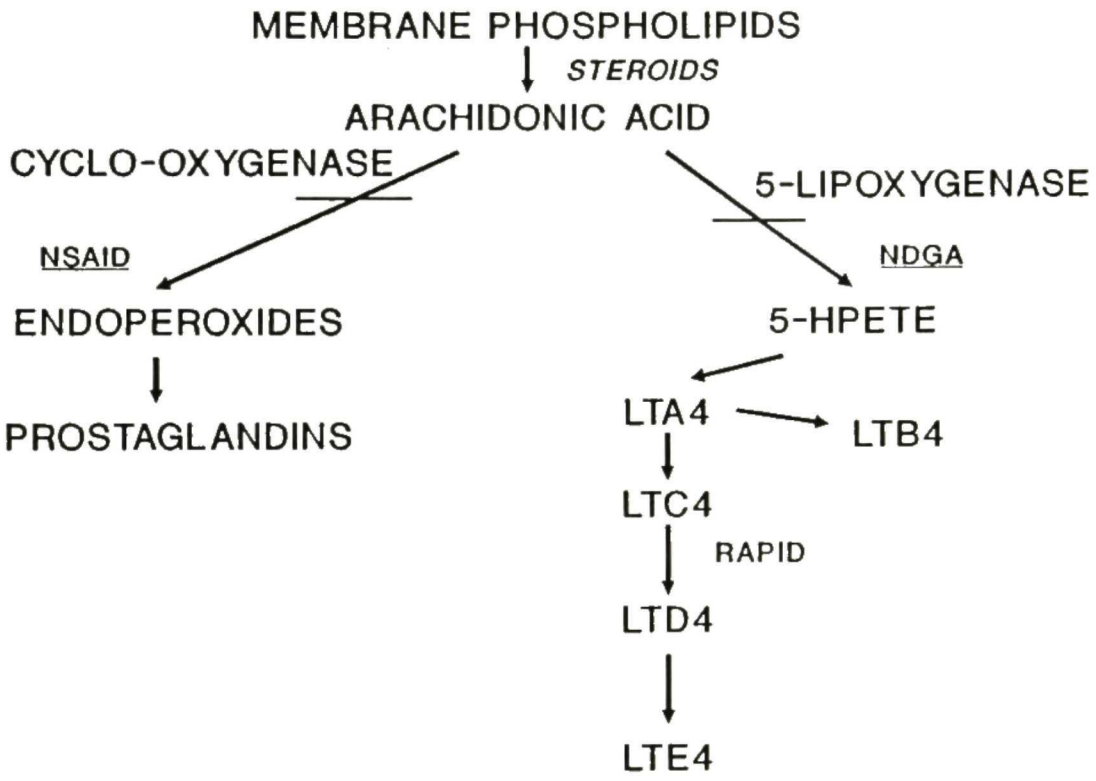
## Arachidonic Acid Cascade

Enzymatic oxidation of arachidonic acid (AA) in membrane phospholipids leads to a multitude of biologically potent compounds, which are referred to collectively as eicosanoids (Figure 2). Among these products are the prostaglandins (PG) and the leukotrienes (LT). AA is cleaved from cell membranes by the action of phospholipase $A_2$ in response to a variety of stimuli. The cleavage of AA is considered to be the rate

limiting step in the formation of eicosanoids since free AA is rapidly metabolized by the oxidative enzymes. Once freed from the cell membrane, the AA may be acted upon by the cyclo-oxygenase (CO) enzyme, or under specific conditions, the lipoxygenase (5, 12, or 15-LO) enzymes. The first product of the CO pathway is an unstable cyclic endoperoxide $PGG_2$, which proceeds to $PGH_2$. $PGH_2$ is the common intermediate for $PGE_2$, $PGF_{2\alpha}$ and $PGI_2$. In certain cell types, AA may be metabolized to the LT by the 5-LO enzyme to yield 5-hydroperoxy-eicosatetraenoic acid (HPETE) and its degradation products 5-hydroxy-eicosatetraenoic acid (HETE) and $LTA_4$. $LTA_4$ may then be transformed to $LTB_4$ by an epoxide hydrolase or to $LTC_4$ by glutathione-S-transferase. The sequential metabolism of $LTC_4$ yields $LTD_4$, $LTE_4$ and $LTF_4$. In those cells expressing the 5-LO enzyme, there is a requirement for the activation of the enzyme in order for the reaction to proceed (Soberman et al., 1986). In contrast, CO does not require activation and its activity is dependent solely on the availability of free AA (Miyamoto et al., 1976).

The arachidonic acid cascade may be manipulated by the inhibition of its metabolic enzymes. The non-steroidal anti-inflammatory drugs (NSAID), such as aspirin (ASA) and indomethacin (INDO), prevent PG synthesis by inhibiting the CO enzyme. ASA acetylates the enzyme but the mechanism of action of INDO is unknown. Corticosteroids block all known pathways of eicosanoid metabolism by preventing the cleavage of AA from the cell membrane. Nordihydroguaiaretic acid (NDGA) has been used prototypically as an inhibitor of the 5-lipoxygenase. However, NDGA is considered a "non-selective" inhibitor of the 5-LO enzyme since at higher concentrations it can inhibit other lipoxygenases as well as thromboxane

Figure 2. The arachidonic acid cascade. Sequential enzymatic oxidation of arachidonic acid can lead to the production of prostaglandins (PG) or leukotrienes (LT). Non-steroidal anti-inflammatory drugs (NSAID) inhibit the cyclo-oxygenase enzyme and nordihydroguaiaretic acid (NDGA) inhibits the 5-lipoxygenase enzyme.


MEMBRANE PHOSPHOLIPIDS
STEROIDS
ARACHIDONIC ACID
CYCLO-OXYGENASE
5-LIPOXYGENASE
NSAID
NDGA
ENDOPEROXIDES
5-HPETE
PROSTAGLANDINS
LTA4
LTB4
LTC4
RAPID
LTD4
LTE4

synthesis (Walker et al., 1980). Recently, specific LT receptor antagonists have been synthesized. One such antagonist, WY48,252 (1,1,1-trifluro-N-[3-(2-quinolinylmethoxy)phenyl]methane sulfonamide, Wyeth Laboratories), has been shown to be an orally active, potent and selective antagonist for the $LTD_4$ receptor (Chang et al., 1988).

The gastrointestinal tract has been shown to have a substantial capacity to synthesize eicosanoids (Dreyling et al., 1986). However, the specific cell types associated with the formation of the eicosanoids in the gastric mucosa have not yet been clearly identified. PG are produced by several types of mucosal cells including parietal and mucus cells. There is some disagreement however as to whether the major product of mucosal cells is $PGE_2$ or $PGI_2$. PG are released in response to a variety of stimuli such as gastric distention, acid and ethanol. In addition, there is a measurable basal release. In contrast, LT are thought to be released from mast cells and neutrophils, two cell types known to play a role in allergic and inflammatory reactions. Thus, this cellular source would be consistent with the low basal release of LT under normal conditions. $LTC_4$ has been shown to be released in response to ethanol (Peskar et al., 1986) and high concentrations of acid (Osada et al., 1990). In summary, PG appear to be released in response to chemical and mechanical stimuli while LT appear to be released only in response to chemical stimuli.

**Visceral Afferent Innervation**

The gastrointestinal tract is extensively innervated by both the parasympathetic and sympathetic divisions of the autonomic nervous system

(ANS). Efferent fibers in the vagus (parasympathetic) and spinal (sympathetic) nerves allow the central nervous system to exert an influence on gastrointestinal function, while afferent fibers in both vagal and spinal nerves carry information from the gastrointestinal organs to the brain. The differences in the function of these two types of afferents are reflected in significant differences in their central and peripheral projections, receptive fields and in the peptides they elaborate.

Figure 3 illustrates the functional anatomy of a spinal sensory afferent neuron. In general, sensory neurons have a single process which arises from the cell body and divides into a peripherally and a centrally directed fiber. A peripheral terminal functions as a receptor and when stimulated sends an impulse which travels in three directions. The impulse first travels back to the mucosa via another peripheral terminal (1). The impulse also travels via collaterals which synapse with prevertebral ganglia (2) and finally, the impulse travels to the CNS (3).

As can be seen in figure 4, the cell bodies of vagal afferents lie in the nodose ganglion (ND) and the cell bodies of the spinal afferents lie in the dorsal root ganglia (DRG). The central processes of vagal afferent fibers terminate in the nucleus of the solitary tract (NTS) in the brainstem and pass to the periphery in the vagus nerve. The central processes of spinal afferent fibers terminate in the dorsal horn of the spinal cord and pass to the periphery in the main sympathetic nerve.

Until the 1950s, the vagus nerve was considered to be primarily a motor nerve. It was the work of light microscopists who revealed that afferent fibers were present in the vagus in far greater numbers than

Figure 3. Functional anatomy of a spinal afferent sensory neuron. Structure 1 represents a peripheral terminal which travels back to the mucosa. Structure 2 represents a collateral to prevertebral ganglia. Structure 3 represents the central terminal. From Mayer et al., 1988.

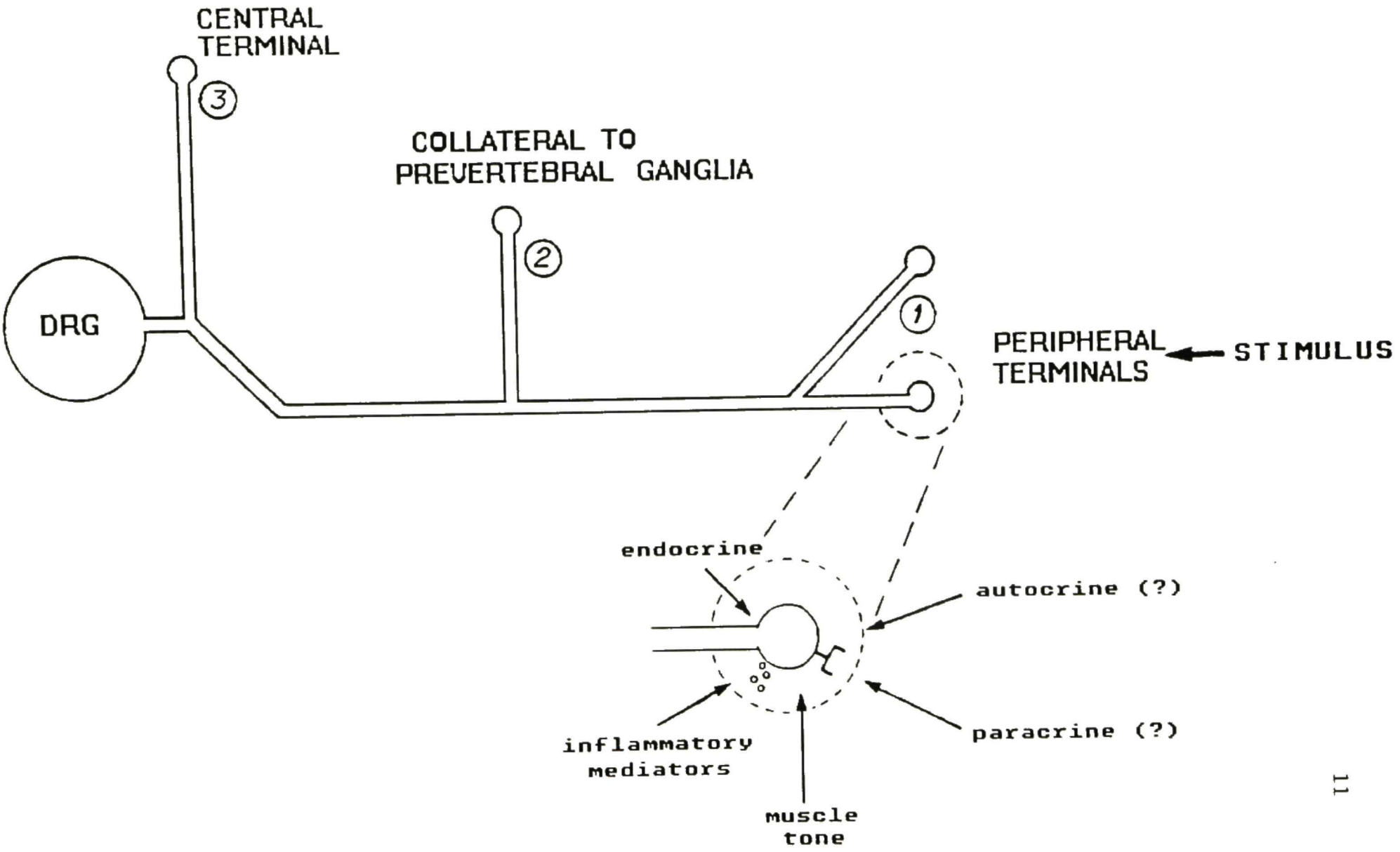
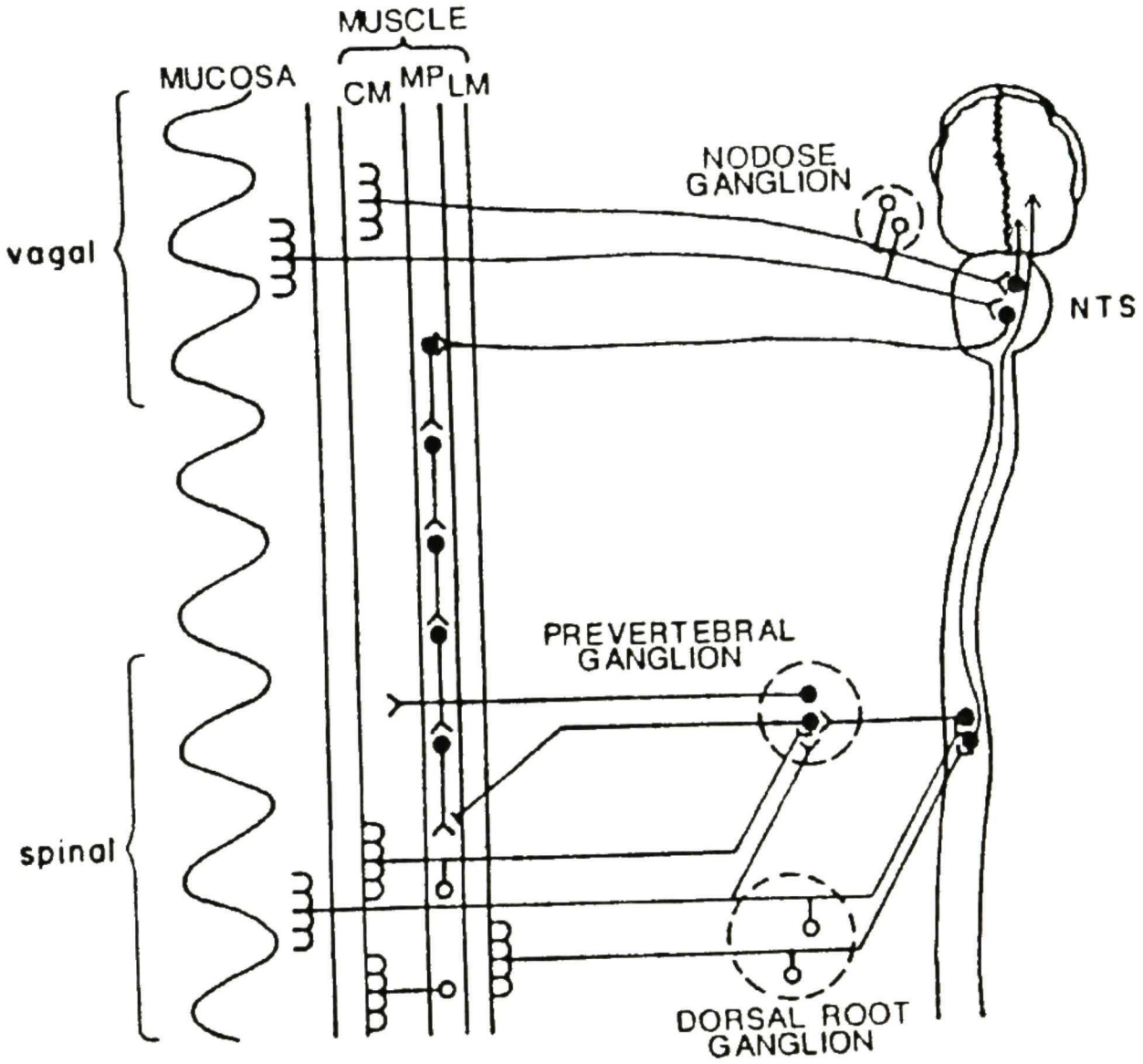

CENTRAL
TERMINAL
3
COLLATERAL TO
PREVERTEBRAL GANGLIA
2
DRG
1
PERIPHERAL
TERMINALS
STIMULUS
endocrine
autocrine (?)
inflammatory
mediators
paracrine (?)
muscle
tone

Figure 4. Innervation of the gastrointestinal tract. CM = circular muscle, LM = longitudinal muscle, MP = myenteric plexus, NTS = nucleus of the solitary tract. From Mayer, E.A. and H. Raybould, 1990.


MUSCLE
MUCOSA
CM
MP
LM
NODOSE GANGLION
vagal
NTS
PREVERTEBRAL GANGLION
spinal
DORSAL ROOT GANGLION

efferent fibers. In fact, the afferent to efferent ratio has been estimated to be as high as 9:1 (Roman and Gonella, 1987). Recent data from electronmicroscopy studies confirm that sensory fibers predominate numerically in the vagus nerve. In contrast, in the splanchnic nerve, spinal afferents account for only 10-20% of fibers. The abdominal vagus innervates the stomach and these afferent fibers show a sensitivity to a variety of stimuli including mechanical, chemical and thermal. Receptors sensitive to mucosal stimuli have been described in the stomach. Mechanically sensitive mucosal receptors are especially sensitive to stroking of the mucosa. However, they are relatively insensitive to distension, contraction, or compression, except when distortion of the mucosa occurs. Because the majority of mechanosensitive mucosal receptors also respond to various chemicals, either applied to the lumen or in the blood, they are considered to be "polymodal" receptors (Grundy and Scratcherd, 1989). Anatomical studies have been unable to identify receptor endings in and outside the wall of viscera suggesting that sensory receptors do not show any morphological specialization and thus are generally considered to be free nerve endings. In contrast to their vagal afferent counterparts, spinal afferents are thought to have their receptive fields predominately in the mesenteric or in the peritoneal ligament. However, some spinal afferents have multivisceral fields; that is, afferent information from different organs is encoded by the same afferent neuron (Grundy and Scratcherd, 1989).

## Neurochemistry Of Visceral Afferents

Immunocytochemical methods have demonstrated many biologically active peptides in vagal and spinal afferents. It has been shown that while there is a great similarity in peptide immunoreactivity within nerve cell bodies, there appear to be significant differences in the localization of neuropeptides between vagal and spinal afferents. For example, 85-95% of spinal afferents to the stomach contain calcitonin gene related peptide (CGRP). However, only 5% of gastric vagal fibers seem to contain CGRP and colocalized substance P (SP) (Dockray et al., 1989). Moreover, immunohistochemical methods have shown that neuropeptides are localized in neurons with other neuropeptides and with classical neurotransmitters such as acetylcholine, noradrenalin, and serotonin (Mayer and Raybould, 1990).

A subpopulation of primary sensory neurons that contain neuropeptides is also characterized by a selective sensitivity to capsaicin, the noxious ingredient of red peppers. Functional and morphological evidence has shown that the gastrointestinal tract is innervated by spinal and vagal capsaicin-sensitive afferent nerve fibers (Fitzgerald, 1983; Russel and Burchiel, 1984). Used chronically, capsaicin is a neurotoxin that is highly specific for nonmyelinated afferent nerve fibers. Thus, when administered in high doses to neonatal animals, capsaicin causes a permanent degeneration of these neurons (Jancso et al., 1977). Used acutely in low doses, capsaicin has been shown to cause the release of SP. Capsaicin-sensitive afferents have been implicated in gastric mucosal protection against ulcerogenic factors such

as indomethacin, cysteamine and ethanol (Holzer and Sametz, 1986). In a study by Holzer and Lippe (1988), stimulation of afferent nerve endings by intragastric capsaicin protected against ethanol-induced damage of the gastric mucosa. Acute sympathetic and parasympathetic denervation, or pretreatment of rats with the sympathetic neuron-blocking drug guanethidine, or parasympatholytic drug, atropine, did not affect the protective effect of capsaicin. These observations imply that afferent nerve-mediated protection of the gastric mucosa does not involve the autonomic nervous system, but may be primarily the result of a local neural mechanism in the stomach.

## Role Of Autonomic Nervous System In Defense Mechanisms

The ANS has long been recognized as an important intermediate between the central nervous system and gastric function (Brooks, 1977). Afferent fibers have been shown to be involved in the reflex regulation of gastrointestinal function. For example, the enterogastric reflex involves signals from sensory endings in the intestinal mucosa which travel to celiac and mesenteric ganglia which, via efferent fibers to the stomach wall can influence the rate of gastric emptying. However, the role of the ANS in defense mechanisms of the gastric mucosa is not well defined. Vagotomy has been demonstrated to be effective in the protection of the gastric mucosa against ASA + HCl (Brodie and Chase, 1967). In addition to having anti-secretory effects, it has been suggested that vagotomy may also stimulate defensive mechanisms in the gastric mucosa such as inhibition of histamine and serotonin release (Rovati et al., 1982). In an elegant series of experiments, Foschi et al. (1986,1989)

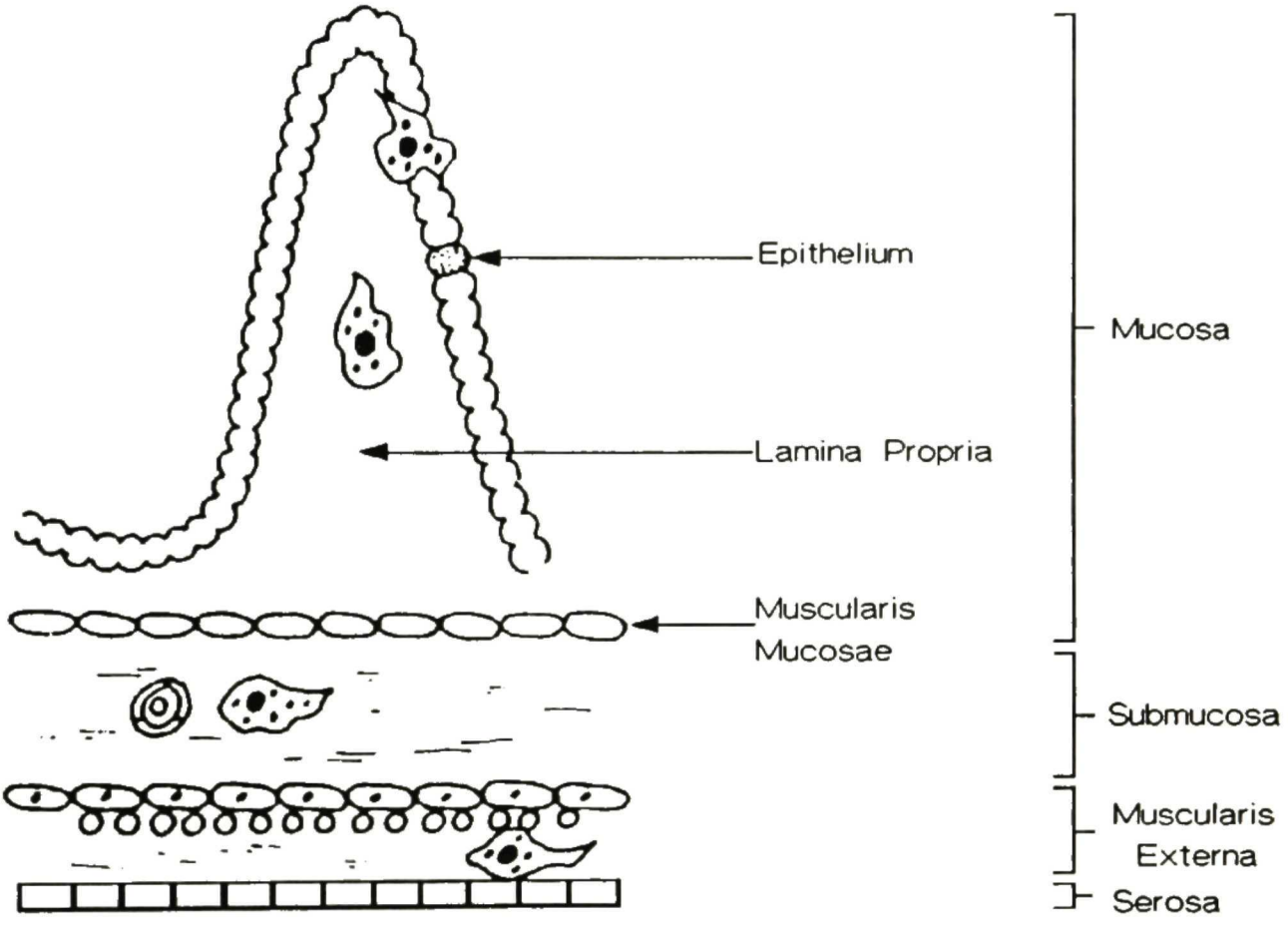
demonstrated that the vagus nerve possess both damaging and protective effects. In addition, Foschi's group demonstrated that atropine and vagotomy protect the gastric mucosa via different mechanisms. Their different actions could be attributable to the fact that the vagus has several components, not just cholinergic drive, which were involved in damage/protection of the gastric mucosa. Specifically, Foschi et al. postulated that when a noxious agent such as ethanol stimulates the afferent vagal nerve it triggers two different effects. One effect is vagally-mediated and is responsible for the damage to the gastric mucosa. The second effect is celiac-mediated and allows anticholinergics or mild irritants to have a protective effect. The first stimulation lead to acetylcholine release and makes the mucosa more responsive to the damaging effects of ethanol and acid back diffusion. This effect could be abolished by the administration of anticholinergics such as atropine. However, vagotomy was shown not be protective but to increase the damage to the gastric mucosa by ethanol, a consequence of the removal of the celiac-mediated (protective) effect.

**Mast Cells and Nerves**

As is illustrated in figure 5, mast cells are easily found in the mucosa, submucosa, muscularis externa and serosa (Saaverdra-Delgado and Metcalf, 1983). Mast cells have been seen within the epithelium in direct content with intestinal contents (Strobel et al., 1981) and are particularly numerous in the lamina propria of the mucosa. They are also found in the submucosa near blood vessels and lymphatics. The mediators that mast cells release can be divided into two categories, specifically

Figure 5. Histological organization of the digestive tract. Mast cells are found in the mucosa, lamina propria and the submucosa. (From Saavedra-Delgado, A.M. and D.D. Metcalf, 1983)

# HISTOLOGIC ORGANIZATION OF THE DIGESTIVE TRACT



those that are preformed and stored within the cytoplasmic granules and those that are newly synthesized following cell activation. Mediators that are preformed include histamine, serotonin, some proteinases and lysosomal enzymes. Mediators synthesized following cell activation include arachidonic acid metabolites such as the prostaglandins, leukotrienes, platelet activating factor, and adenosine (Schwartz and Austen, 1984). While the function of mast cell in the gastrointestinal mucosa remains unclear, mast cell-derived mediators have been suggested to induce localchanges in vasopermeability, stimulate mucous production, increase muscle contraction, stimulate pain fibers and recruit inflammatory cells (Saavedra-Delgado and Metcalf, 1983).

It is now clear that mast cells do not represent a homogeneous population but that extensive morphological and functional heterogeneity exists among mast cell populations. Thus, mucosal mast cells (MMC) differ from connective tissue mast cells not only in their morphologic and staining characteristics, but also in their response to secretagogues (Lee et al., 1985). For example, MMC have been shown to contain less histamine than other mast cells. Also, compound 48/80, which causes degranulation in other mast cells, has no effect on MMC (Saavedra-Delgado et al., 1984).

There is increasing evidence for both microanatomic associations and physiological interactions between peripheral nerves and MMC. Newson et al. (1983) provided ultrastructural evidence for bouton formation between MMC and enteric nerves in the rat ileum. More recent studies have shown that in rats, there is an intimate microanatomic association between MMC and nerves in the lamina propria (see figure 5), a subdivision of the mucosa (Stead et al., 1987). Moreover, both SP and CGRP immunoreactivity

could be demonstrated in the nerves apposed to mast cells (Stead et al., 1987 and 1988). There is also evidence that MMC are closely apposed to nerves in the human gastrointestinal mucosa providing a basis for potential paracrine and neurocrine interactions between mast cells and nerves in the gastrointestinal mucosa (Stead et al., 1989). Thus, it is likely that in the submucosa nerves which are closely apposed to mast cells cause the mast cell to degranulate and release its contents. LT released from the mucosal mast cells may subsequently have an effect on the gastric mucosa.

In conclusion, it is known that the gastrointestinal mucosa has the capacity to generate eicosanoids in response to noxious stimuli such as ethanol. It remains to be determined if exposure of the gastric mucosa to physiological but potentially noxious luminal contents can stimulate the generation of eicosanoids. Another question that remains to be answered is what mechanisms play a role in the generation of eicosanoids after exposure of the gastric mucosa to noxious luminal contents.

# RATIONALE

The gastric mucosa has the capacity to synthesize eicosanoids. Both leukotrienes and prostaglandins, oxidation products of arachidonic acid, have potent effects on gastric function. For example, prostaglandins and leukotrienes decrease gastric hydrogen ion secretion, increase bicarbonate ion secretion and increase mucus secretion. However, the prostaglandins and leukotrienes have opposing effects on gastric mucosal blood flow; prostaglandins are known to increase blood flow whereas leukotrienes are known to decrease blood flow. While both leukotrienes and prostaglandins are released in response to agents that damage the gastric mucosa, such as high concentrations of acid or ethanol concentrations over 40%, the pattern of leukotriene and prostaglandin synthesis in response to physiological concentrations of acid or bile is not well defined.

This study was designed to determine if the gastric mucosal response to potentially noxious luminal stimuli involves the selective induction of eicosanoid generation, and to examine the mechanisms that may be involved in this generation.

During times of peak acid secretion, acid concentration may be as high as 150 mM. Bile, which is physiologically the most important endogenous barrier breaker, is normally kept out of the stomach by the pylorus. However, bile can be refluxed from the duodenum to the stomach and, the concentration of bile salts found in the gastric juice of man with gastric ulcer has been reported to be as high as 7.4 mM. In the present study, the concentration of the acid loads was 50 and 100 mM HCl

and the concentration of the bile salt load was 5 mM. These concentrations are well within physiological levels. The results from these studies demonstrated that there was a selective induction of eicosanoid generation in response to potentially noxious luminal stimuli.

This selective induction may be the result of different cellular sources of the prostaglandins and the leukotrienes. It has been suggested that leukotrienes are generated by mucosal mast cells and that prostaglandins are generated by surface epithelial cells. Moreover, recent investigations have demonstrated a close association between mucosal mast cells and nerves. Therefore, in the next series of experiments, the role of sensory afferent nerves in the selective induction of gastric mucosal eicosanoid generation in response to physiological concentrations of acid or bile was investigated. Lidocaine, a local anesthetic, was used to inhibit sensory afferent nerve. Removal of the sensory input to the mucosal mast cell would be expected to decrease leukotriene generation in response to acid and bile while having no effect on prostaglandin generation.

The results of this research support the hypothesis that luminal contents that are potentially noxious to the gastric mucosa can selectively induce the generation of gastric mucosal eicosanoids. The data presented here suggests that sensory afferents may mediate the generation of leukotrienes but not prostaglandins. This finding adds new understanding of the role of eicosanoids in the formation of gastric ulcers and an extension of this work may aid in the understanding of the pathophysiology of gastric ulcer and other inflammatory processes such as ulcerative colitis.

# MATERIALS AND METHODS

## Animals

Male unanesthetized rhesus monkeys (Macaca mulatta) weighing 3-8 kg were adapted to primate restraining chairs and housed in closed, ventilated, lighted booths between 0900 and 1200 hours. The monkeys were trained to accept a 12 French double lumen nasogastric ventrol Levin tube (National Catheter, Mallinckrodt, Arglye, New York; bore 4 mm; wall thickness, 1 mm). The experiments were conducted after an overnight fast. Proper positioning of the tube in the most dependent part of the stomach was verified by demonstrating that after injecting 15 ml of water into a previously emptied stomach, the total volume could be recovered (Dubois et al., 1977a). The monkeys were habituated to the intubation procedure once a week for a month and subsequent studies were performed with approximately the same frequency.

## Drug Administration

Animals were treated either by intravenous (iv), subcutaneous (sc) or intragastric (IG) drug administration. Intravenous drug administration was via an Angiocath (21 gauge) placed in the animal's saphenous vein and secured to animal's leg with tape. Extension tubing, a syringe and a Harvard constant infusion pump were used to administer the drug. For sc drug administration, a 21 gauge butterfly was placed under the skin of the animal's leg and the infusion set-up was the same as described above. For IG administration, drugs were injected into the stomach via the nasogastric tube.

**Blood Pressure Determination**

Blood pressure was monitored in some studies using a Hewlett-Packard 7758 recorder interfaced with a programmed electro-sphygmomanometer (PE-300, Narco Biosystems, Inc., Houston, TX). A pediatric blood pressure cuff containing a Kortokoff microphone was placed on the animals's upper left arm over the brachial artery. Using a pressure sound amplifier, the onset of the audible pulse was used to determine systolic blood pressure, and the disappearance of the audible pulse, diastolic blood pressure. By knowing chart speed, heart rate was determined by counting the number of pulse pressure waves during a given time period. A minimum of three measurements was taken during each treatment. These measurements were then averaged to obtain mean values for systolic and diastolic blood pressures.

**Gastric Function Studies**

A $^{99m}$Tc-DTPA (diethylenetriaminepentaacetic acid) dilution technique previously described and validated in monkeys and humans (Dubois et al., 1977a & b) was used to determine simultaneously gastric emptying and secretion. Animals were studied during a fasting period and at 5, 10 and every 10 minutes thereafter following the intragastric administration of an 80 ml fluid load. All loads were infused at 37° C because temperature can influence gastric emptying. The control load had a pH of 7.4, while the acid load was either 50 mM HCl or 100 mM HCl with a pH around one. The maximum intragastric hydrogen ion concentration in man has been shown to be 150 mM (Granger, 1988), so 50 and 100 mM HCl loads are well within physiological concentrations. The 5 mM bile salt (Sigma

Chemical Co., St. Louis, MO) load was a mixture of equal parts of the unconjugated bile salts sodium cholate ($pK_a$ 5.2) and sodium deoxycholate ($pK_a$ 6.30) at a pH of 5.2. Samples of gastric juice were centrifuged and aliquots of the clear supernatant were counted in a autogamma counter (Ultragamma, LKB Instruments Inc., Maryland). Intragastric volumes of fluid ($V_1$, $V_2$ ...) and amounts of $^{99m}$Tc-DTPA were determined using the marker dilution principle. (George, 1968; Hildes and Dunlop, 1951) Gastric emptying (g) was determined for each interval (t) between two dilutions (P=concentration of marker), assuming that emptying of water is a first order (exponential) process during short intervals and using the equation:

$$g=-\log_e (P_2 / P_1)/t$$

Fractional emptying rate (FER) was determined at 5, 10 and every 10 minutes thereafter, for a total of 60 minutes, following the intragastric administration of the load, which provides a mild distention stimulus. The gastric emptying response to the load is particularly strong in the first 10 minutes after the administration of the load. During this interval, approximately 30 to 50% of the load may be emptied in controls, making this interval unique relative to the remainder of the five 10-minute intervals. Thus, two measurements of gastric emptying in the first 10 minutes permits a more accurate assessment of the changes which take place during this interval. Gastric emptying in controls often returns to fasting values by 20 to 30 minutes after the load. For these reasons, the initial 5- and 10-minute intervals were averaged to indicate changes which occur during the initial response to the load and these values were reported as early (0-10 min) FER. The subsequent five 10-

minute intervals were then averaged. Alternatively, the mean of the initial emptying and the subsequent emptying were also averaged to determine one postload mean value per animal. The mean (± S.E.M) of these postload values (0-10 min and 0-60 min) was then calculated for each study. Net rate of fluid output ($R_v$) was determined for the corresponding interval assuming that it remained constant over the given interval and using the equation:

$$R_v = [V_2 - V_1 \times \exp(g \times t)] \times g/[1 - \exp(-g \times t)]$$

Intragastric fluid volumes were then recalculated taking into account the first estimate of fractional emptying and fluid output, which were in turn recalculated (Dubois et al., 1977a).

Hydrogen ion output was measured by end-point titration to pH 7.4 with 0.02 N NaOH (Radiometer Titration Assembly, Oberlin, OH). The concentrations of sodium ($Na^+$) and potassium ($K^+$) ions in the gastric juice were measured using a flame photometer (IL Model 443, Lexington, MA) and chloride ($Cl^-$) ion concentration was determined using an amperometric titration method (Corning 920 M, Medford, MA). The intragastric mass of each ion ($I_1, I_2 \ldots$) was determined by multiplying the intragastric ion concentration by the corresponding intragastric volume. The net rate of each ion output ($R_I$) was then calculated using the equation:

$$R_I = [I_2 - I_1 \times \exp(g \times t)] \times g/[1 - \exp(-g \times t)]$$

The calculations were performed using a locally developed program and PDP-10 computer (Division of Computer Research Technology, National Institutes of Health, Bethesda, MD). The assumptions are: (1) the marker is neither absorbed, degraded, secreted nor acted upon by the stomach, (2) emptying and secretion remain constant over the interval, but may vary

between intervals, (3) the gastric contents and marker can be completely mixed in the one minute mixing interval, and (4) that the gastric contents and marker remain homogeneously mixed (Dubois et al., 1977a). This method is based on the original contribution of Hildes and Dunlop (1951). However, in contrast to their method, the present technique allows for correction of emptying and secretion during the one minute marker-dilution interval and can be applied during fasting as well as following a water load (Dubois et al., 1977a, b).

### Tissue Collection

Upon completion of each marker dilution study, the nasogastric tube was removed. In some studies, the monkeys were then anesthetized with an intramuscular injection of 5-10 mg/kg ketamine hydrochloride (Vetalar, Morris Plains, NJ) and underwent gastroscopy using an Olympus GIF-P2 pediatric endoscope (Olympus Corp. of America, New Hyde Park, NY). First, a visual examination was made of the overall appearance of the mucosa in both the antrum and fundus. The number and size of any lesions were noted and scored subsequently by two investigators who were unaware of the experimental treatment. Scoring was done using a modification of the method described by Lanza et al. (1980, Table I). After visual inspection of the mucosa, biopsies were taken from both the antrum (n=3) and the fundus (n=3) and placed in ice cold Tyrode's solution to be used for the measurement of $LTC_4$ and $PGE_2$ generation. For histological evaluation, additional biopsies were put in 1) Carnoy's fixative (60 ml absolute ethanol, 30 ml chloroform, 10 ml glacial acetic acid) for 24 hours and subsequently put into formalin for mast cell determination after

embedding, sectioning and staining with toluidine blue (Strobel et al., 1981) and 2) 10% neutral buffered formalin for hematoxylin and eosin staining. The severity of microscopic mucosal damage was evaluated by two independent investigators who were unaware of the treatment group using the classification of Lacy and Ito (1982, see Table II). Damage is based upon a score of 0 for no damage, I for damage to luminal surface cells only, II for damage to luminal cells and gastric pit cells and III for damage to all cells from luminal surface and gastric gland cells. Only sections that were well orientated and cut perpendicularly to the mucosal surface were graded.

Mast cells were counted in well orientated sections cut perpendicularly to the mucosal surface and in which the muscularis mucosa was intact. Counts were performed on coded slides on an Olympus system microscope, model BH-2 by two investigators who were unaware of the treatment. Lines on the eyepiece used for photography were calibrated against an improved Neubauer hemacytometer. The edge of the line was orientated along the muscularis mucosae and the area comprised 80-100% of the total depth of the mucosa. Although each slide held 3 to 5 cuts of the biopsy, cut at 5 $\mu$m sections, only one cut (3 to 4 fields) was counted. The MMC count was expressed as MMC/0.46 $mm^2$.

TABLE I

Scale of Evaluation of Macroscopic Gastric Injury

| Grade | Description |
|---|---|
| 0 | No injury |
| 1 | Reddening or 1 discrete submucosal hemorrhage |
| 2 | 2 or more submucosal hemorrhages |
| 3 | 1 erosion, $\leq$ 1 mm in diameter |
| 4 | 2-5 erosions, 1 mm in diameter |
| 5 | >5 erosions, or $\geq$ 1 linear erosion |

From Lanza et al., 1980.

## Table II

### Classification and Description of Microscopic Damage to Gastric Mucosa

| Score | Description of Damage |
|---|---|
| 0 | None; all gastric mucosal cells appeared intact and had normal shape, location, appearance and density. Surface mucus cells are columnar to cuboidal with varying amounts of apical mucous granules. Gastric pits are of expected depth and the gastric glands are comprised of intact mucous neck, parietal, chief and endocrine cells. Only rare cells undergoing exfoliation. |
| I | Luminal surface cell damage only; surface mucous cells on the surface are vacuolated, have pyknotic nuclei, lightly stained cytoplasm, or lysed cytoplasm, some cell exfoliation is present. Gastric pit cells undamaged. |
| II | Luminal surface and gastric pit cell damage; in addition to extensive luminal surface cell damage the cells lining the gastric pits are also disrupted and exfoliated. The gastric gland cells are not damaged. |
| III | All cells from luminal surface and gastric gland cells damaged; beneath the damaged surface and gastric pits, cellular damage is evident in the gastric glands. Parietal cells with lucent cytoplasm common. Numerous exfoliated cells and whole layers of necrotic superficial epithelium present. Lamina propria often seen in direct continuity with stomach lumen. |

From Lacy and Ito, 1982.

Eicosanoid Generation

Ex vivo generation of LT was determined using a modification of the method of Dreyling et al. (1986). Biopsies obtained by endoscopy were washed three times in ice-cold modified Tyrode's buffer. They were then placed in 1 ml of prewarmed (37°C) and oxygenated (95% $O_2$/5% $CO_2$) modified Tyrode's solution containing 10 mmol/l glutathione and 100 mM serine borate to prevent the conversion of $LTC_4$ to $LTD_4$, and incubated at 37°C in a heat block for 20 min. After 20 min, the incubation medium was removed and immediately placed in a -70 °C freezer and replaced by an equal volume of identical buffer containing 10 μg/ml of the calcium ionophore, A23187 (Calbiochem, San Diego, CA). The calcium ionophore increases the ability of divalent ions, such as calcium, to cross biological membranes by forming stable complexes with the ions, rendering them more soluble. The increase in calcium stimulates phospholipase activity, thereby increasing intracellular free arachidonic acid (Knapp et al., 1977). At the end of the 20 min incubation time, the Tyrode's was removed to terminate the generation of LT. All incubates were stored at -70°C until determination of LT and PG was performed. The biopsy tissue was frozen for subsequent protein determination by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL). $LTC_4$ was determined by RIA (Dupont/New England Nuclear, Boston, MA). In this assay, a radioactive ($^3H$) antigen competes with a nonradioactive antigen (samples, standards) for a fixed number of antibody binding sites. When unlabeled antigen from samples or standards and a fixed amount of tracer (labeled antigen) are allowed to react with a constant and limiting amount of antibody, decreasing amounts of tracer are

bound to the antibody as the amount of unlabeled antigen is increased. To separate the antibody-antigen complex from the free antigen, 500 $\mu$l of activated charcoal (0.5 % charcoal Norit A, Amend Drug and Chemical Company, Irvington, NJ) was used to adsorb the free tracer. The tubes were centrifuged and the supernatant was suspended in Ready-Solv (Beckman, Fullerton, CA) scintillation cocktail and counted in a liquid scintillation counter. After counting has been completed, the concentration of $LTC_4$ in the samples is determined from the standard curve (0.0125 to 0.8 ng $LTC_4$/0.1 ml). Samples that contain amounts of $LTC_4$ at the high end of the standard curve have less counts per minute than samples that have amounts of $LTC_4$ on the low end of the standard curve. This assay has less than 1% cross reactivity with compounds such as PG, AA, linoleic acid and palmitic acid. The percent cross reactivity with 11-trans-$LTD_4$ and $LTD_4$ is 60.5 and 55.3 % respectively.

$PGE_2$ was determined by an enzyme immunoassay kit (Cayman Chemical Company, Ann Arbor, MI). The assay is based on the competition between free $PGE_2$ tracer for limited specific rabbit antiserum binding sites. The rabbit antiserum-$PGE_2$ complex (free or tracer) then binds to the mouse monoclonal anti-rabbit antibody that is electrostatically bound to the wells of a 96-well microtiter plate. The plate was then washed and Ellman's Reagent was added to the well. The reagent contains acetylthiocholine, which is cleaved by the acetylcholinesterase tracer to give free thiocholine. The thiocholine then reacts with the dimer of 5-thio-2 Nitrobenzoic acid the second component of Ellman's reagent, producing a distinct yellow color which is measurable within 30 minutes. The density of the color, which is determined spectrophotometrically, by

absorbance at 415 nM in a microplate reader (Bio-Tek model E1309, Winooski, Vermont) is proportional to the amount of free $PGE_2$ present in the well during the incubation. Precise $PGE_2$ concentrations in the samples were determined by comparison with a standard curve of $PGE_2$ provided with the kit.

**Data Analysis**

The statistical significance of differences observed in measurements of gastric function were evaluated using a three-factor analysis of variance (treatment, time and monkey) with repeated measures on the last two factors, (Kirk, 1968) followed by a test (Newman-Keuls or orthogonal) designed to determine the significance of differences among multiple means using the program LDU-040 (K.L. Dorn), and an IBM 370 computer (Division of Computer Research and Technology, National Institutes of Health, Bethesda, MD). The statistical significance of differences observed between the histological scores and eicosanoid generation were evaluated by either a students t-test if there were two groups for comparison, or a one way ANOVA followed by a Bonferroni test if there were more than two groups. Values of $P < 0.05$ were regarded as significant.

# EXPERIMENTAL RESULTS

## The Effect of Administration of Exogenous Leukotriene $D_4$ on Gastric Emptying and Secretion

The effect of $LTD_4$ on gastric and hemodynamic function was investigated in conscious rhesus monkeys following an intravenous infusion of saline or $LTD_4$ (0.1 or 0.2 $\mu$g/kg/min). Blood pressure, heart rate, gastric emptying, fluid and ion outputs were determined after an 80-ml water load. Figure 6 summarizes systolic and diastolic blood pressure and heart rate. Neither dose of $LTD_4$ altered systolic or diastolic blood pressure or heart rate. When compared to control, neither dose of $LTD_4$ caused macroscopic damage to the gastric mucosa as determined by the macroscopic damage scores (0.17 $\pm$ 0.17 vs. 0.4 $\pm$ 0.3, 0.7 $\pm$ 0.3 control, 0.1 $\mu$g/kg/min $LTD_4$, 0.2 $\mu$g/kg/min $LTD_4$ respectively). In addition, as determined by the microscopic damage scores, neither dose of $LTD_4$ caused microscopic damage to the gastric mucosa (1.25 $\pm$ 0.34 vs. 1.17 $\pm$ 0.62, 0.70 $\pm$ 0.30 control vs. 0.1 $\mu$g/kg/min $LTD_4$, 0.2 $\mu$g/kg/min $LTD_4$ respectively).

Depicted in figure 7, panel A, is the effect of $LTD_4$ on the mean fractional emptying rate. Although the 0.2 $\mu$g/kg dose of $LTD_4$ decreased mean fractional emptying rate by 31%, this decrease was not significantly different when compared to control. This is further illustrated in figure 7, panel B, which shows that neither dose of $LTD_4$ produced a significant change in the percent of the load remaining.

In contrast to the lack of a significant effect on gastric emptying, both doses of $LTD_4$ decreased hydrogen ion output (Figure 8).

Summarized in Table III is the effect of $LTD_4$ on fluid, sodium, potassium and chloride ion secretion. Both doses of $LTD_4$ significantly decreased sodium ion secretion, but had no effect on fluid, potassium or chloride output or concentration.

Figure 6. The effect of $LTD_4$ administered intravenously (0.1 and 0.2 $\mu$g/kg/min) on blood pressure (mmHg) and heart rate (beats/minute). Values from both doses of $LTD_4$ were averaged to obtain one mean $\pm$ S.E.M. for $LTD_4$ treatment. Control values are the means $\pm$ S.E.M. of 5 animals and $LTD_4$ values are the means $\pm$ S.E.M. of 9 animals.

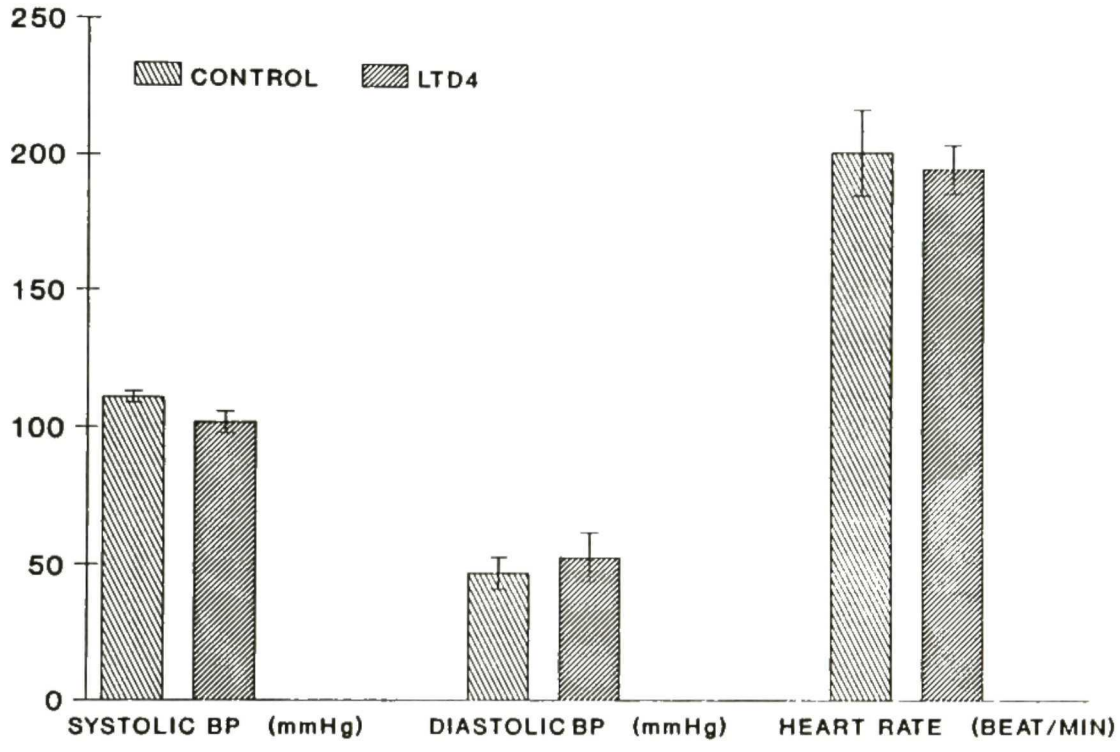
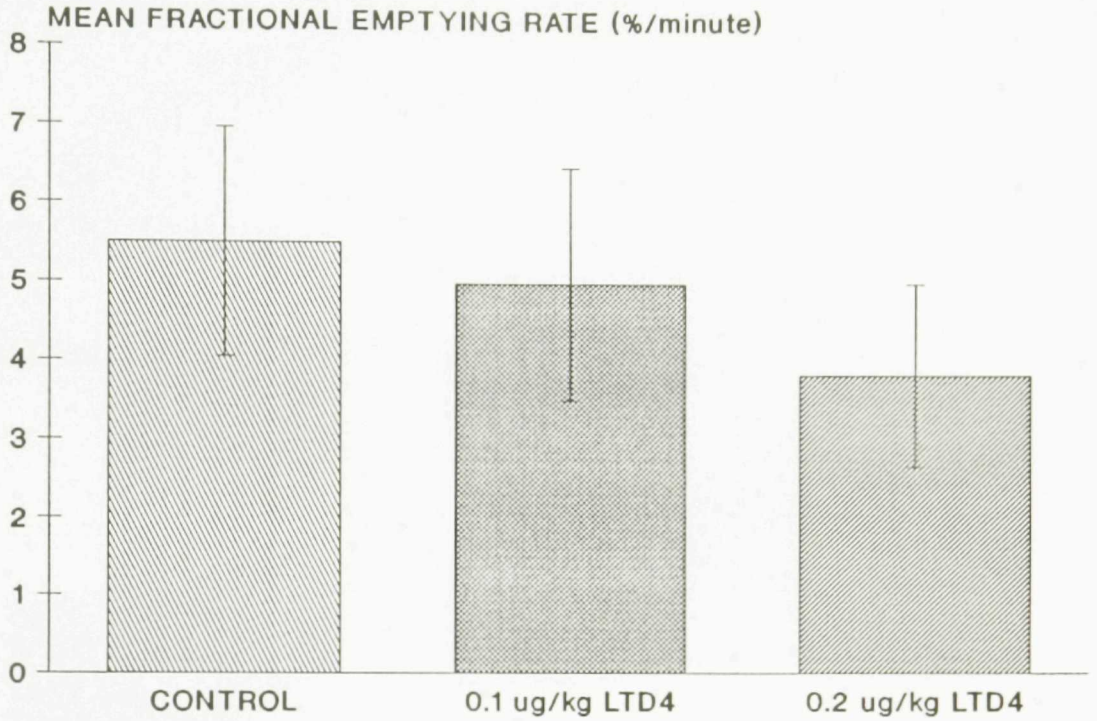

250
200
150
100
50
0
CONTROL
LTD4
SYSTOLIC BP (mmHg)
DIASTOLIC BP (mmHg)
HEART RATE (BEAT/MIN)

Figure 7. The effect of intravenous administration of $LTD_4$ (0.1 or 0.2 μg/kg/min) on mean fractional emptying rate (Panel A). Values are the means ± S.E.M. of five animals. Panel B illustrates the effect of intravenous administration of $LTD_4$ on the percent of the load remaining. Values are the means of 5 animals.

PANEL A




PANEL B

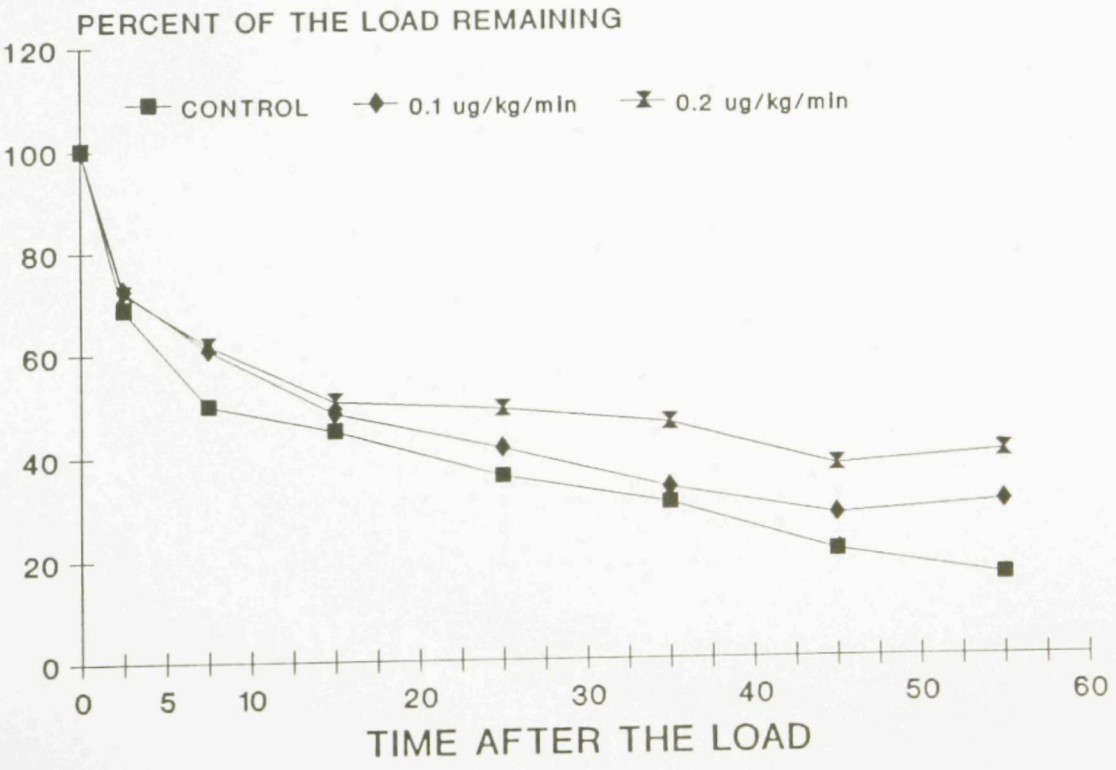
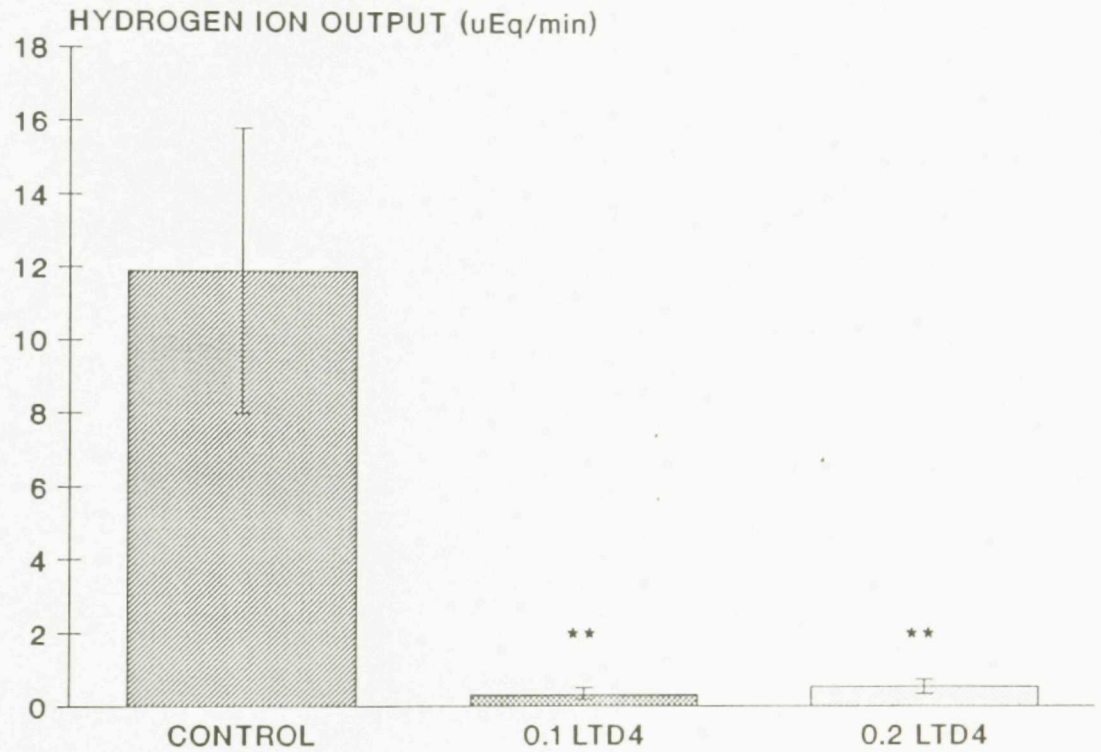


Figure 8. The effect of intravenous administration of exogenous $LTD_4$ on hydrogen ion output. Values are the means $\pm$ S.E.M. of 5 animals, ** $P < 0.001$.


HYDROGEN ION OUTPUT (uEq/min)
18
16
14
12
10
8
6
4
2
0
**
**
CONTROL
0.1 LTD4
0.2 LTD4

## TABLE III

### Effect of $LTD_4$ on Fluid and Ion Output and Ion Concentration

| | CONTROL | $LTD_4$ 0.1μg/kg/min | $LTD_4$ 0.2μg/kg/min |
|---|---|---|---|
| Fluid Output (ml/min) | 0.56 ± 0.11 | 0.56 ± 0.14 | 0.46 ± 0.10 |
| $H^+$ Output μEq/min | 11.9 ± 3.9 | 0.3 ± 0.1 ** | 0.52 ± 0.52 ** |
| $Na^+$ Output μEq/min | 28.5 ± 4.7 | 17.8 ± 5.2 ** | 19.9 ± 3.9 ** |
| $Na^+$ Concn μEq/ml | 86.0 ± 12.5 | 54.7 ± 9.9 * | 51.8 ± 11.3 * |
| $K^+$ Output μEq/min | 7.1 ± 1.5 | 6.9 ± 1.8 | 5.3 ± 1.0 |
| $K^+$ Concn μEq/ml | 20.7 ± 3.7 | 22.6 ± 4.7 | 13.3 ± 2.5 |
| $Cl^-$ Output μEq/min | 51.1 ± 9.6 | 50.7 ± 7.5 | 43.5 ± 4.9 |
| $Cl^-$ Concn μEq/ml | 112.0 ± 8.9 | 110.4 ± 14.0 | 108.3 ± 8.3 |

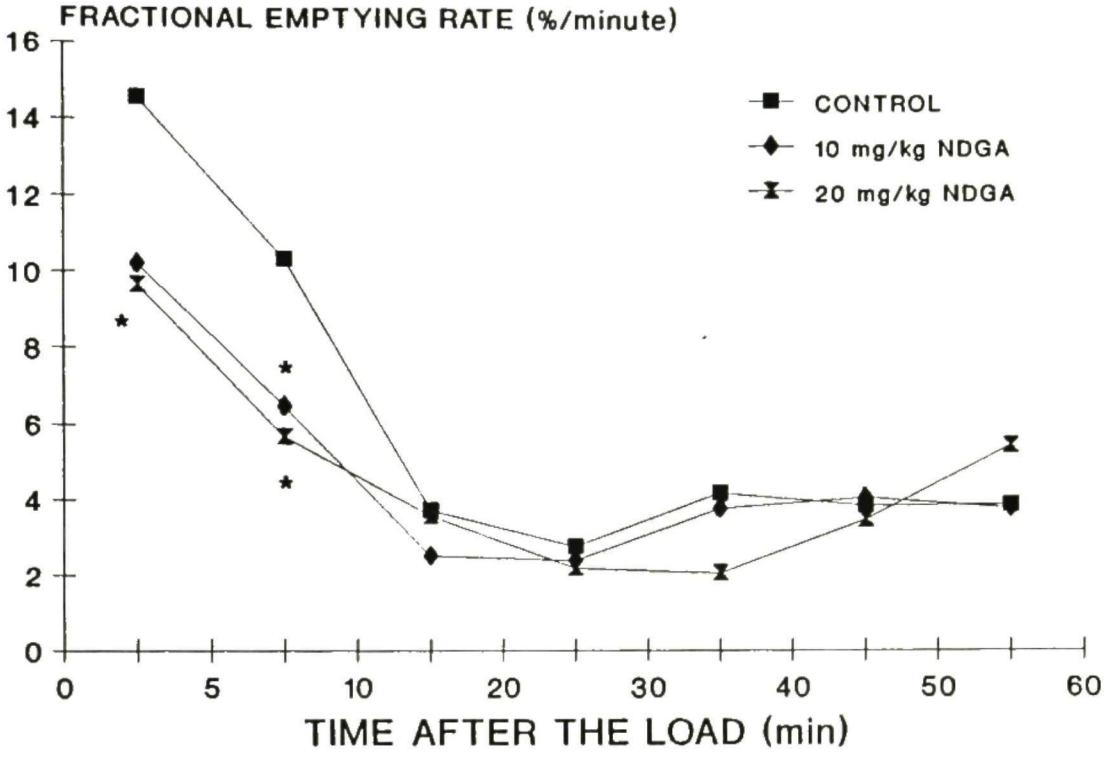
Values are means ± S.E.M. of measurements in 5 monkeys. * $p < 0.05$ and ** $p < 0.001$ when compared to control, using a three factor analysis of variance (treatment, time and monkey) with repeated measures on the last two factors, followed by a t-test designed to determine the differences among multiple means. Concn = concentration.

**The Effect of Administration of a 5-lipoxygenase Enzyme Inhibitor on Gastric Emptying and Secretion**

The effect of inhibition of endogenous LT synthesis was investigated using NDGA, an inhibitor of the 5-lipoxygenase enzyme. Animals received a 2 ml subcutaneous injection of either vehicle (20 mEq $NaHCO_3$) or NDGA (10 or 20 mg/kg). When compared to control (4.50 $\pm$ 0.72 %/min), neither dose of NDGA altered mean (0-60 min) FER (4.49 $\pm$ 0.94, 3.68 $\pm$ 0.65 %/min, n=6). As shown in figure 9, panel A, both doses of NDGA produced a dose-dependent decrease in FER from 30% to 40% between 0 and 10 minutes after the load. Despite the decrease in FER induced by the 20 mg/kg dose of NDGA, there was no significant effect on the percent of the load remaining (Figure 9, panel B). The effect of NDGA on fluid and ion secretion is summarized in Table IV. Neither dose of NDGA significantly altered fluid or ion secretion.

Figure 9. The effect of NDGA, a 5-LO inhibitor, on gastric emptying. **Panel A.** The effect of NDGA on fractional emptying rate plotted over the 60 minute postload period. Values are the means of 6 animals, * $P < 0.05$ vs control at the same time. **Panel B.** The effect of NDGA on percent of the load remaining in the stomach at the end of each interval. Values are the means of 6 animals.

PANEL A




PANEL B

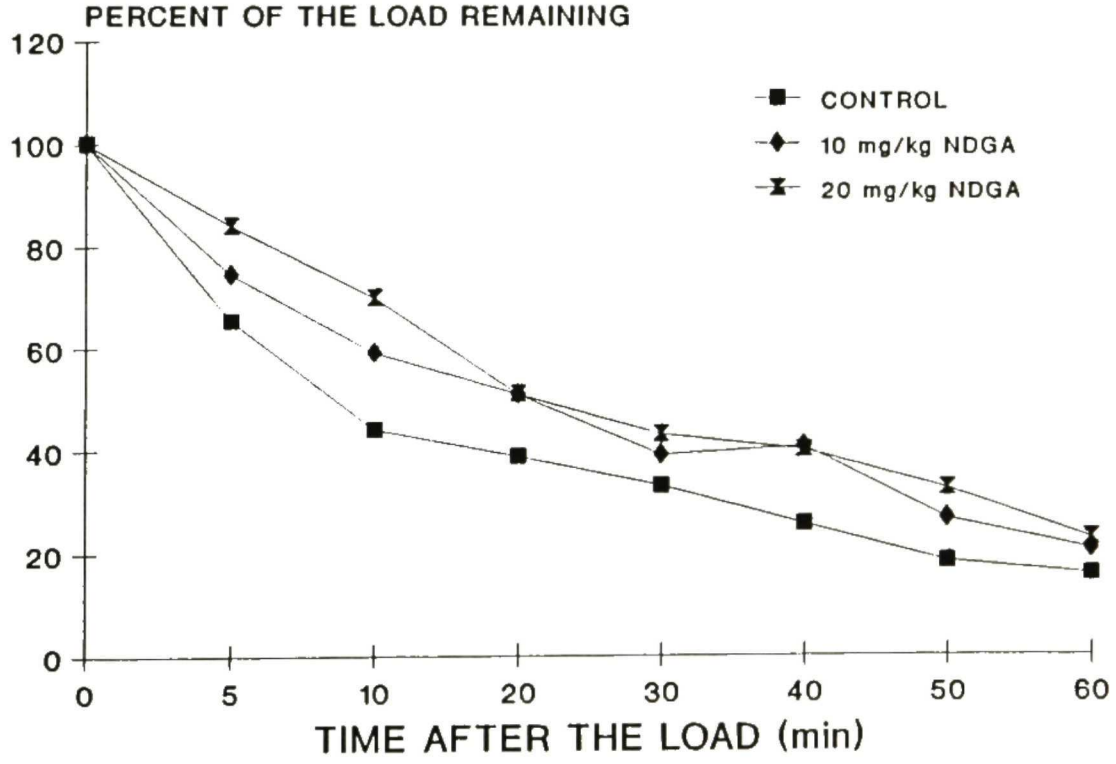


TABLE IV

Effect of NDGA on Fluid and Ion Output and Ion Concentration.

| | CONTROL | NDGA 10 mg/kg | NDGA 20 mg/kg |
|---|---|---|---|
| Fluid Output (ml/min) | 0.22 ± 0.05 | 0.20 ± 0.03 | 0.15 ± 0.02 |
| $H^+$ Output μEq/min | 7.1 ± 3.5 | 7.3 ± 2.9 | 6.7 ± 4.1 |
| $H^+$ Concn μEq/ml | 47.5 ± 18.9 | 34.6 ± 11.0 | 30.6 ± 16.3 |
| $Na^+$ Output μEq/min | 8.8 ± 1.8 | 11.2 ± 1.5 | 10.5 ± 1.1 |
| $Na^+$ Concn μEq/ml | 40.7 ± 6.3 | 54.5 ± 5.4 | 58.7 ± 6.3 |
| $K^+$ Output μEq/min | 3.8 ± 1.4 | 4.5 ± 1.7 | 3.5 ± 1.3 |
| $K^+$ Concn μEq/ml | 18.6 ± 7.0 | 24.9 ± 9.4 | 19.4 ± 7.4 |
| $Cl^-$ Output μEq/min | 29.9 ± 2.9 | 22.6 ± 2.9 | 21.1 ± 3.1 |
| $Cl^-$ Concn μEq/ml | 115.5 ± 10.4 | 106.8 ± 9.1 | 108.9 ± 13.6 |

Values are means ± S.E.M. of measurements in 6 monkeys. Significance was determined using a three way analysis of variance (treatment, time and monkey) with repeated measures on the last two factors, followed by a t-test designed to determine the differences among multiple means. Concn = concentration.

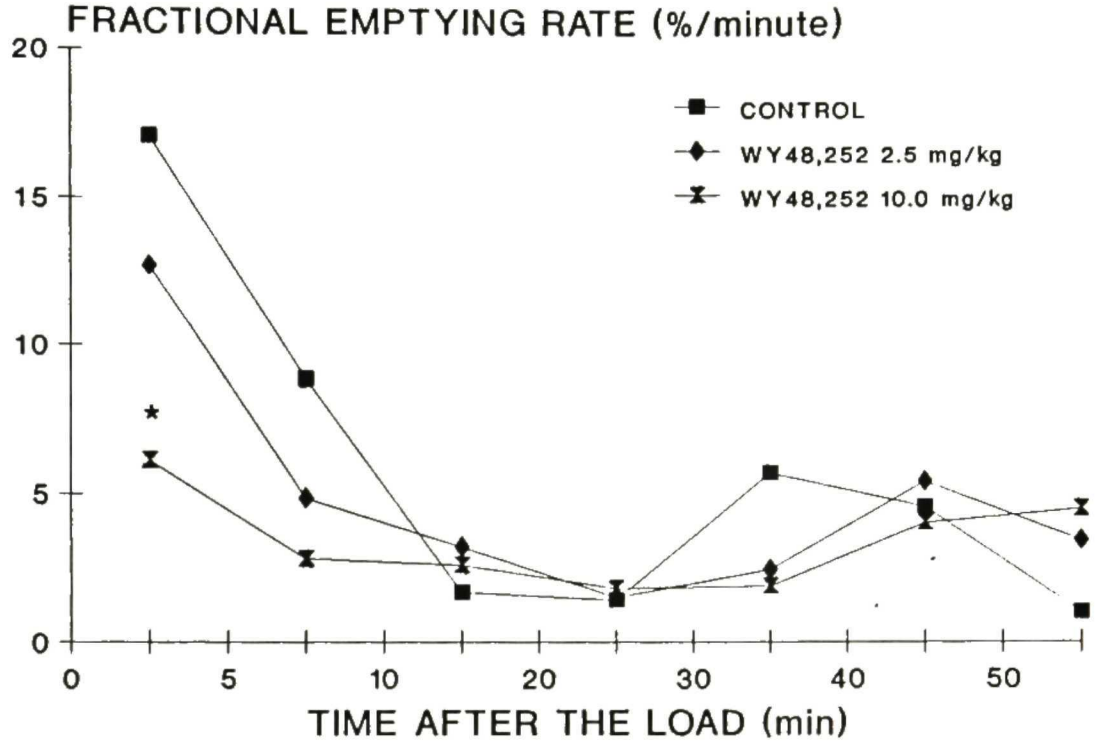
**The Effect of Administration of a Leukotriene $D_4$ Receptor Antagonist on Gastric Emptying and Secretion**

Studies were performed to determine the effect of antagonism of the $LTD_4$ receptor on gastric function. Animals received an intragastric bolus of vehicle (propylene glycol, 3 ml) or an $LTD_4$ receptor antagonist, WY48,252 (2.5 or 10 mg/kg). When compared to control (4.23 $\pm$ 0.59 %/min), neither dose of WY48,252 significantly altered mean FER (4.15 $\pm$ 1.05, 3.22 $\pm$ 0.72 %/min, n=5). Although neither dose of WY48,252 altered mean FER, the 10 mg/kg dose of WY48,252 significantly depressed FER at 5 minutes (Figure 10, panel A). The decrease in FER by the 10 mg/kg dose of WY48,252 produced a significant delay in the emptying of the load from 10 to 40 minutes (Figure 10, panel B).

Table V summarizes the effect of WY48,252 on fluid and ion secretion. Neither dose of WY48,252 significantly altered fluid, or hydrogen, sodium, potassium or chloride ion secretion.

Figure 10. The effect of an $LTD_4$ receptor antagonist, WY48,252, on gastric emptying. **Panel A.** The effect of WY48,252 on mean fractional emptying rate. Values are the means $\pm$ S.E.M. of 5 animals, * $P < 0.05$ when compared to control at the same time interval. **Panel B.** The effect of WY48,252 on percent of the load remaining. Values are the means of 5 animals, * $P < 0.05$ vs control at the same time interval.

PANEL A




PANEL B

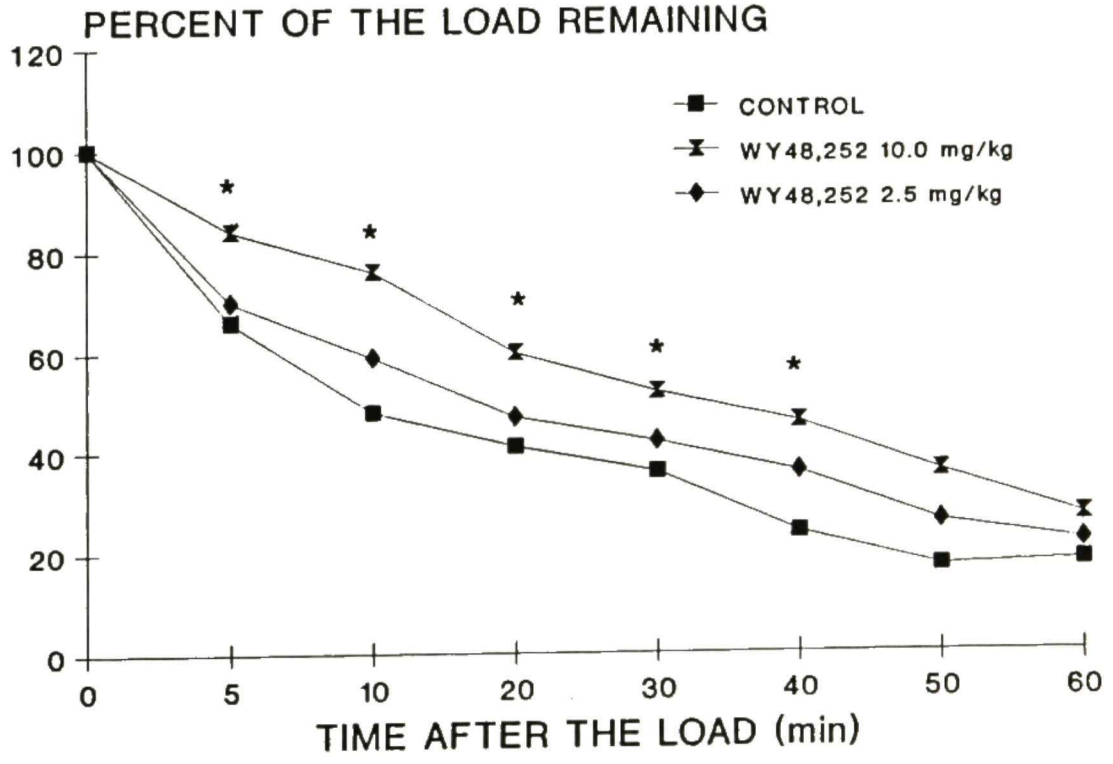


TABLE V

Effect of WY48,252 on Fluid and Ion Output and Ion Concentration.

| | CONTROL | WY48,252 2.5 mg/kg | WY48,252 10 mg/kg |
|---|---|---|---|
| Fluid Output (ml/min) | 0.34 ± 0.11 | 0.23 ± 0.05 | 0.38 ± 0.03 |
| $H^+$ Output μEq/min | 2.9 ± 2.6 | 1.7 ± 1.1 | 2.1 ± 1.1 |
| $H^+$ Concn μEq/ml | 10.9 ± 10.8 | 8.2 ± 5.2 | 8.8 ± 6.0 |
| $Na^+$ Output μEq/min | 23.6 ± 4.3 | 20.4 ± 1.9 | 27.3 ± 2.6 |
| $Na^+$ Concn μEq/ml | 66.0 ± 11.2 | 89.0 ± 11.8 | 65.7 ± 12.2 |
| $K^+$ Output μEq/min | 8.2 ± 7.6 | 5.7 ± 0.51 | 7.6 ± 1.1 |
| $K^+$ Concn μEq/ml | 23.8 ± 2.3 | 24.4 ± 2.9 | 17.3 ± 4.2 |
| $Cl^-$ Output μEq/min | 35.3 ± 7.6 | 37.0 ± 2.8 | 48.6 ± 6.9 |
| $Cl^-$ Concn μEq/ml | 82.4 ± 3.3 | 88.4 ± 4.5 | 84.2 ± 4.2 |

Values are means ± S.E.M. of measurements in 5 monkeys. Significance was determined using a three way analysis of variance (treatment, time and monkey) with repeated measures on the last two factors, followed by a t-test designed to determine the differences among multiple means. Concn = concentration.

**The Effect of Exposure of the Gastric Mucosa to Acid or Bile Salt Load on Gastric Emptying and Mucosal Eicosanoid Generation**

To determine if physiological but potentially noxious agents alter eicosanoid generation in the gastric mucosa, animals received intragastrically one of four loads: water (control), 50 mM HCl, 100 mM HCl or 5 mM bile salts. As is illustrated in figure 11, panel A, only the 5 mM bile salt load significantly decreased mean (0-60 min) FER. However, both the 100 mM HCl and 5 mM bile salt load inhibited FER during the early (0-10 min) interval (Fig 11, panel B). The effect of each of these agents on gastric emptying over time is further illustrated in figure 12 which depicts the percent of the load remaining. When compared to control, neither of the acid loads nor the bile salt load altered the percent of the load remaining. In addition, neither the acid loads nor the bile salt load caused a statistically significant amount of macroscopic (Table VI) or microscopic damage (Table VII). The photomicrographs in figure 13 further illustrate that the acid and bile load did not cause microscopic damage. Shown in panel A is a section from a control animal. Note that the surface epithelial cells are full of mucus and the surface cells form a continuous layer. Panel B is a photomicrograph from an animal that received a 5 mM bile salt load. The surface epithelial cells again are full of mucus and form a continuous layer. Due to the rigorous criteria followed for the MMC counts, many biopsies were deferred from the count due to incorrect orientation or missing muscularis mucosa. Thus, due to the small number of MMC counts in some treatment groups, no statistical analysis was performed. There did not appear to be a difference between control ($5.6 \pm 2.3$ MMC/0.46 $mm^2$, n=3), 100 mM HCl ($5.3 \pm 1.8$ MMC/0.46 $mm^2$,

n=3) or 5 mM bile salt load (1.0 ± 1.0 MMC/0.46 $mm^2$, n=1) MMC counts. Using a subjective approach, there appeared to be a difference in the intensity of the staining of the mast cells with cells from animals that received the acid or bile salt load appearing somewhat lighter. This difference in staining may be due to a partial degranulation of the mast cells.

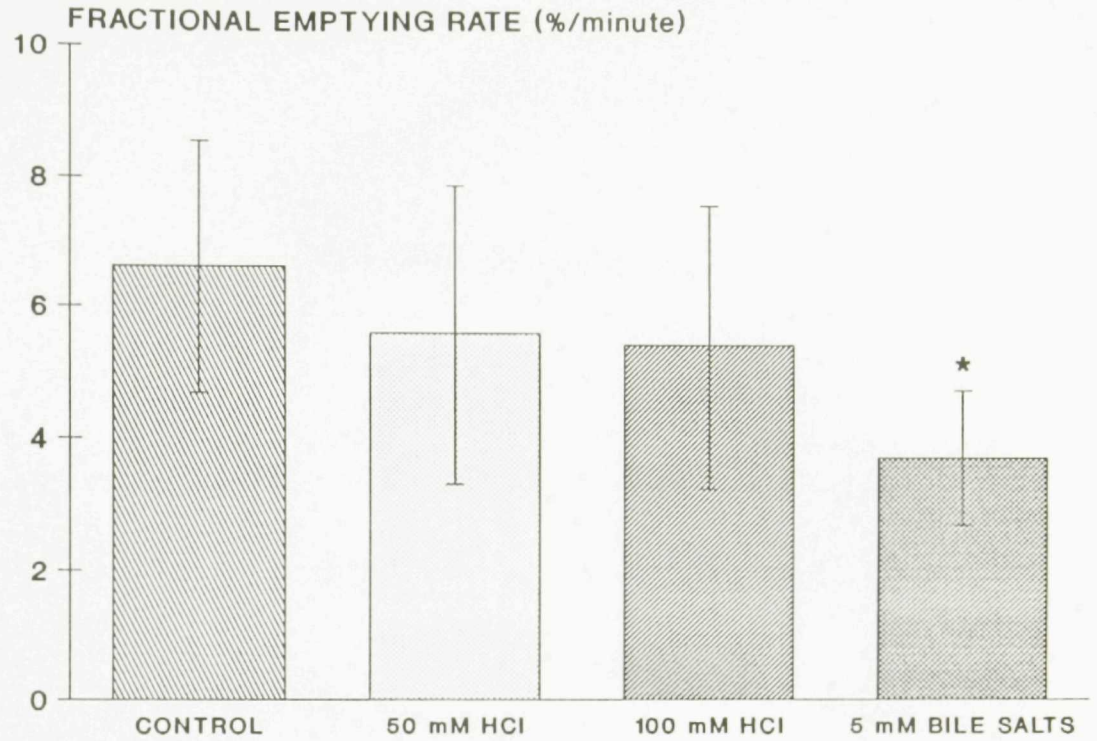
As is shown in figure 14, mucosal $LTC_4$ generation was very low in the antrum (panel A) and undetectable in the fundus (panel B). Exposure of the gastric mucosa to either of the acid loads or the bile salt load increased basal $LTC_4$ generation in both the antrum and fundus. Figure 15 presents $PGE_2$ generation data in the antrum and the fundus. As compared to figure 14, the basal release of $PGE_2$ was a thousand fold higher on a molar basis than the basal release of $LTC_4$ and, in contrast to $LTC_4$, there was a measurable generation of $PGE_2$ after exposure of the gastric mucosa to the water load. In addition, the bile salt load, but not the acid loads, caused a significant increase in basal $PGE_2$ generation.

Figure 16 illustrates the effect of administration of an intragastric load of either acid or bile salt on basal versus calcium ionophore stimulated $LTC_4$ generation. Following calcium ionophore, $LTC_4$ generation was stimulated in control samples. In contrast, mucosal exposure to either acid or bile salts did not produce an additional increase in $LTC_4$ synthesis following stimulation by calcium ionophore alone. As is depicted in figure 17, the mucosal generation of $PGE_2$ was not stimulated by the calcium ionophore and, in fact, appeared to decline with the A23187. These results suggest that the cellular source of $PGE_2$ is not responsive to an influx of extracellular calcium and in fact, the synthesis of $PGE_2$ may

undergo a degeneration in the face of increased calcium (Dreyling et al., 1986). There was no difference between levels of $LTC_4$ and $PGE_2$ in the antrum when compared to the fundus. In addition, no correlation was found between the generation of $PGE_2$ or $LTC_4$ and the change in either early or mean FER.

Figure 11. The effect of administration of an intragastric load of either acid (50 or 100 mM HCl) or 5 mM bile salts on fractional emptying rate. Values are the means ± S.E.M. in 6 animals. **Panel A** illustrates mean fractional emptying rate measured over the 60 minute postload period, * $P < 0.05$ versus control. **Panel B** illustrates fractional emptying rate during the early (0-10 minute) interval, * $P < 0.05$ versus control.

PANEL A




PANEL B

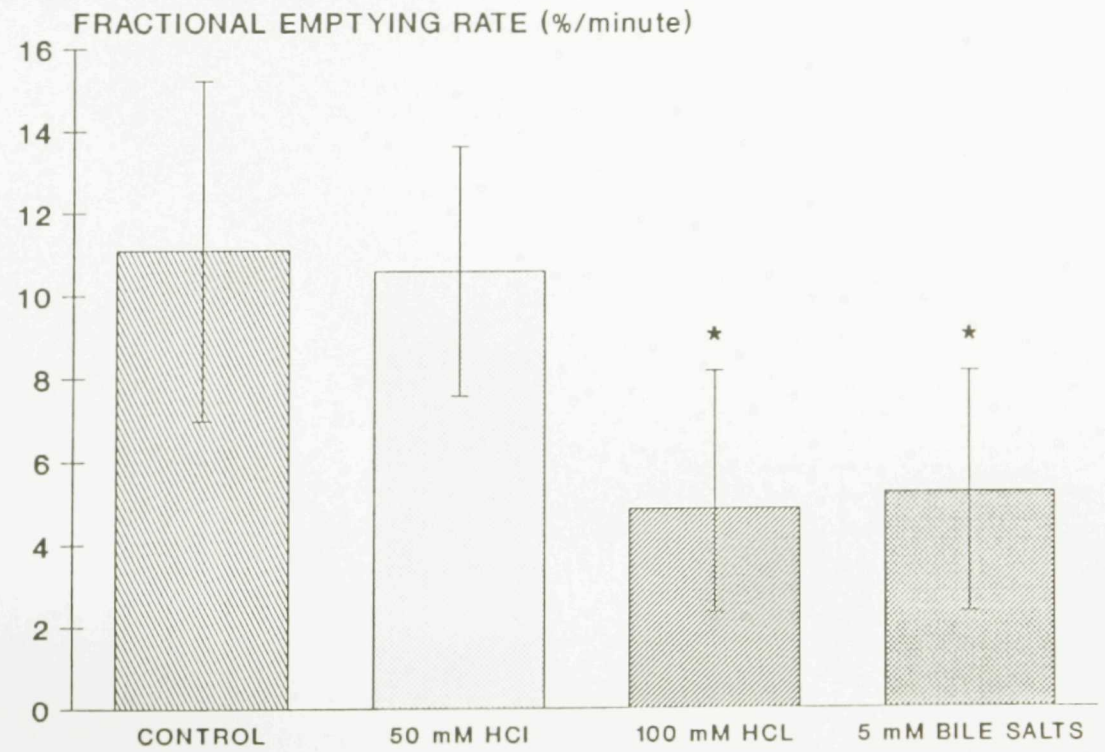
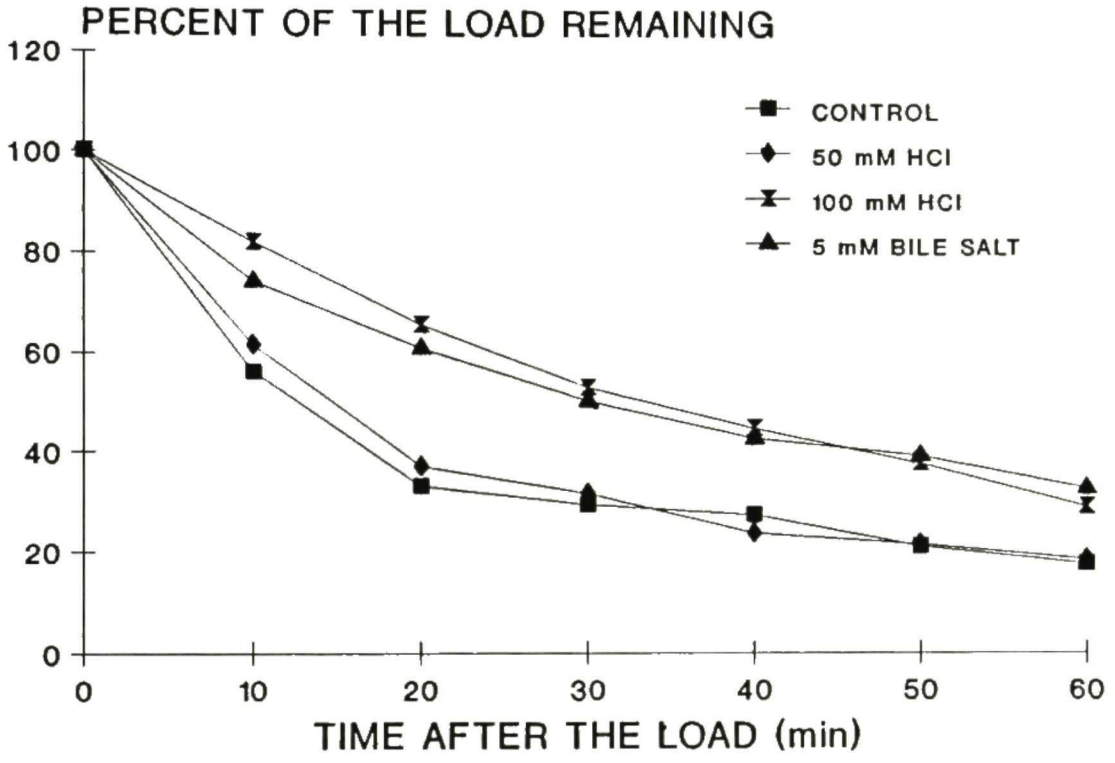


Figure 12. The effect of the administration of an intragastric load of either acid (50 or 100 mM HCl) or 5 mM bile salts on percent of the load remaining. Values are the means of 6 animals.


PERCENT OF THE LOAD REMAINING
120
100
80
60
40
20
0
CONTROL
50 mM HCl
100 mM HCl
5 mM BILE SALT
0
10
20
30
40
50
60
TIME AFTER THE LOAD (min)

## TABLE VI

### MACROSCOPIC DAMAGE SCORES

| TREATMENT | SCORE |
|---|---|
| CONTROL (6) | 0.17 ± 0.17 |
| 50 mM HCl LOAD (5) | 0.80 ± 0.19 |
| 100 mM HCl LOAD (6) | 0.33 ± 0.21 |
| 5 mM BILE SALT LOAD (6) | 0.50 ± 0.22 |

Values are means ± S.E.M. (#) = number of animals evaluated. Antral and fundal scores were averaged to one overall mean.

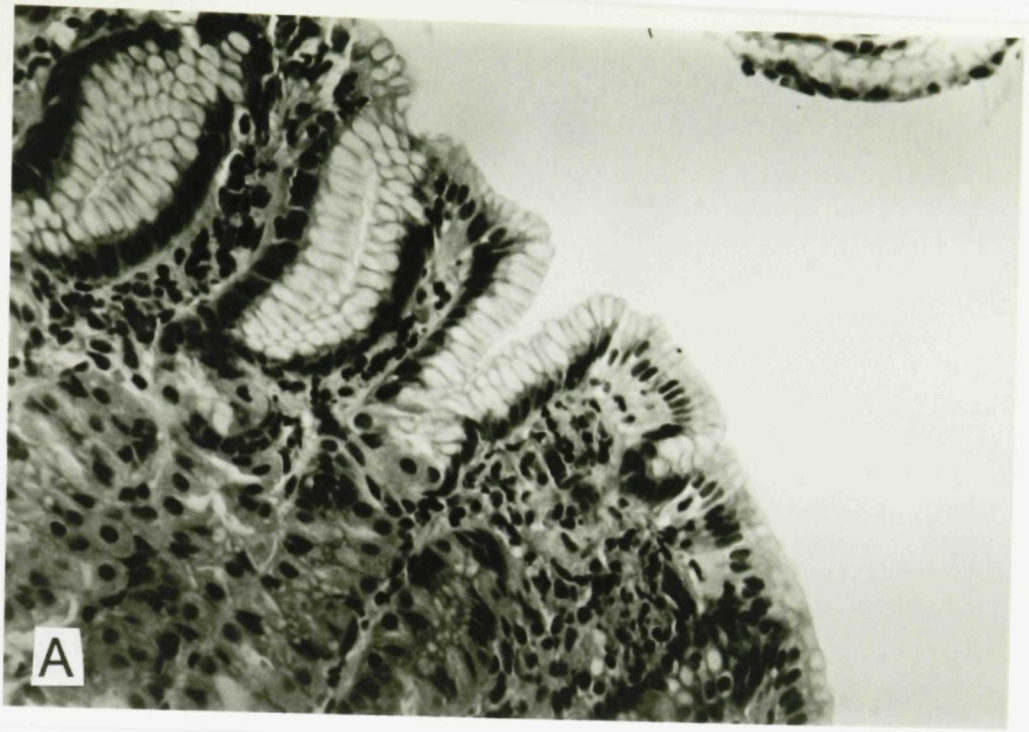
TABLE VII

MICROSCOPIC DAMAGE SCORES

| TREATMENT | SCORE |
|---|---|
| CONTROL (6) | 1.25 ± 0.34 |
| 50 mM HCl LOAD (5) | 1.42 ± 0.46 |
| 100 mM HCl LOAD (6) | 1.81 ± 0.25 |
| 5 mM BILE SALT LOAD (6) | 1.5 ± 0.38 |

Values are means ± S.E.M. (#) = number of animals assayed. Thus, antral and fundal scores were averaged to obtain one overall mean.

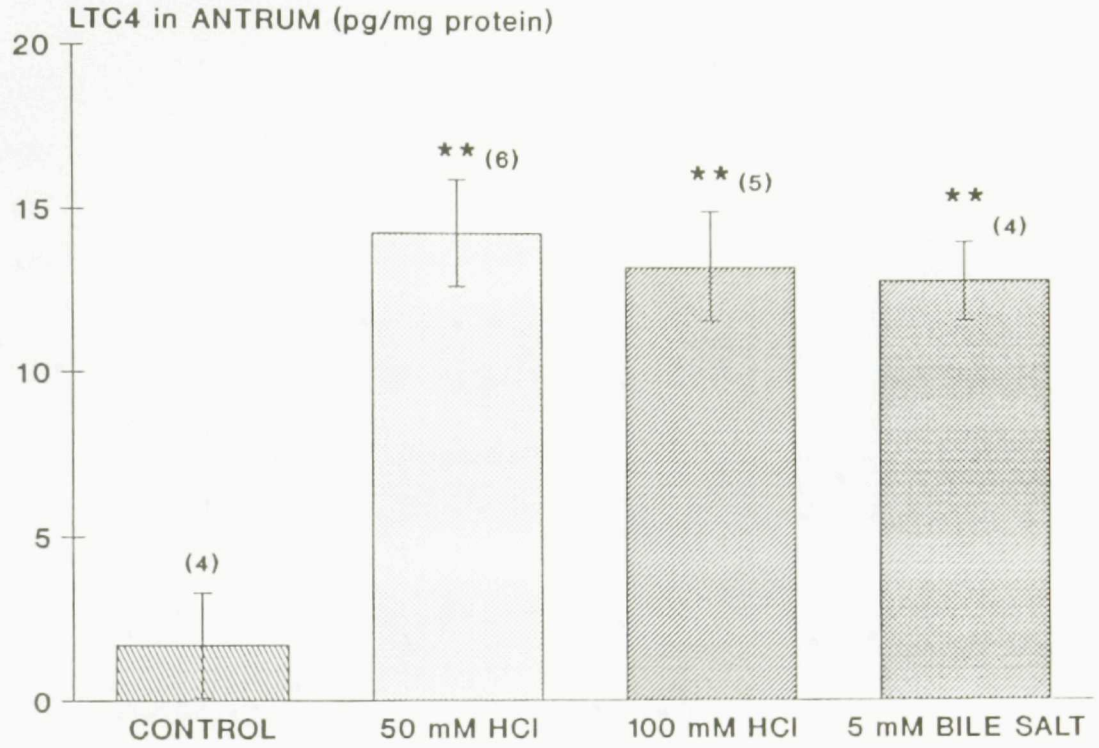
Figure 13. Photomicrographs illustrating hematoxylin and eosin stained gastric mucosal biopsies. **Panel A** illustrates an animal exposed to a water load (control). **Panel B** illustrates an animal exposed to a 5 mM bile salt load. Magnification X 226.


A


B

Figure 14. The effect of administration of an intragastric load of either acid (50 or 100 mM HCl) or 5 mM bile salts on the gastric mucosal generation of $LTC_4$ in the antrum (**Panel A**) or fundus (**Panel B**). Values are means $\pm$ S.E.M. (#) = number of animals, * $P < 0.05$ and ** $P < 0.01$ versus control.

PANEL A




PANEL B

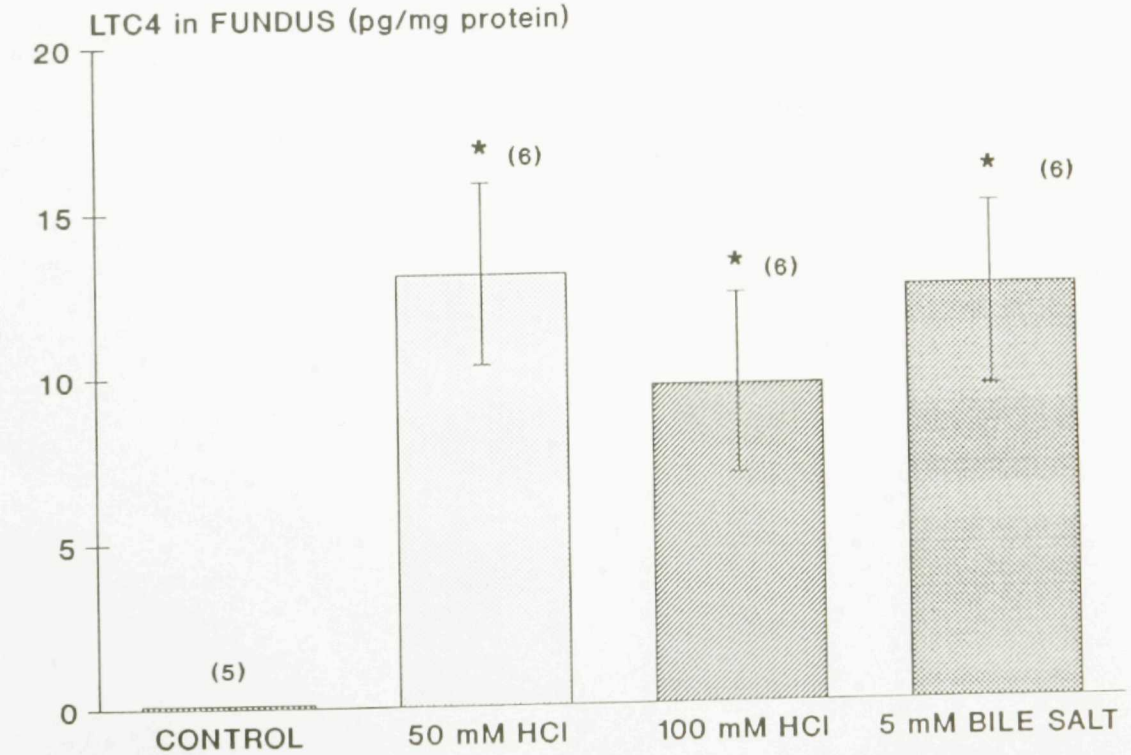
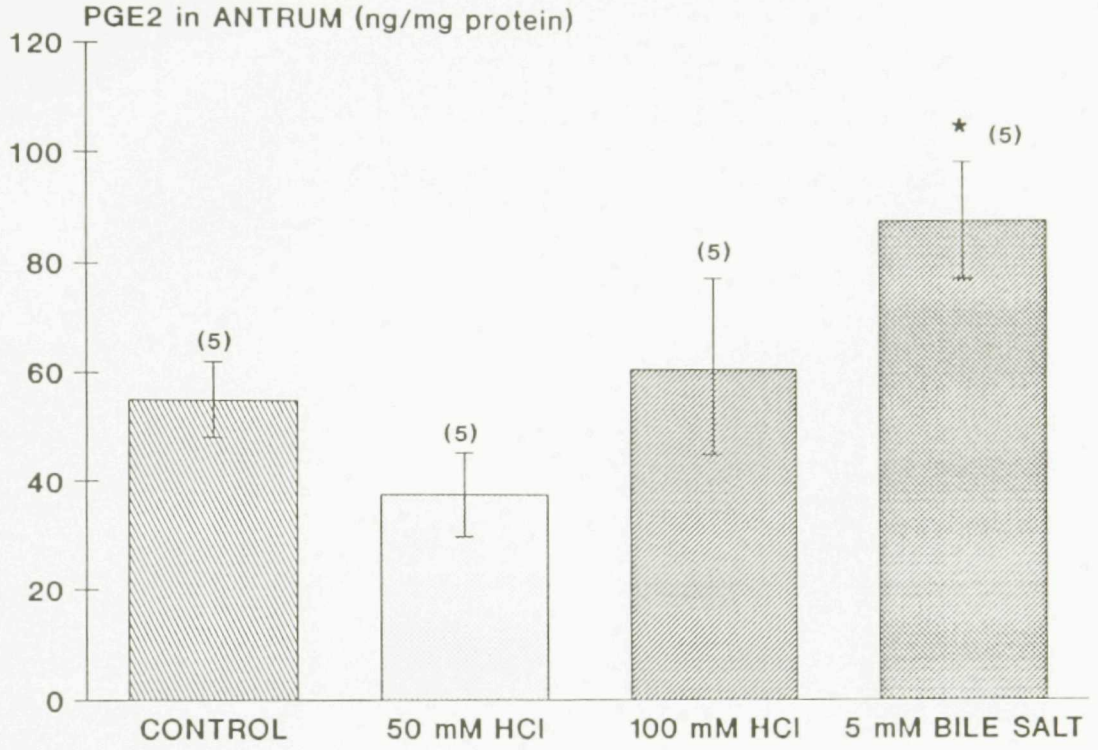


Figure 15. The effect of administration of an intragastric load of either acid (50 or 100 mM HCl) or 5 mM bile salts on gastric mucosal generation of $PGE_2$ in the antrum (**Panel A**) or the fundus (**Panel B**). Values are means $\pm$ S.E.M. (#) = number of animals. * $P < 0.05$ versus control.

PANEL A




PANEL B

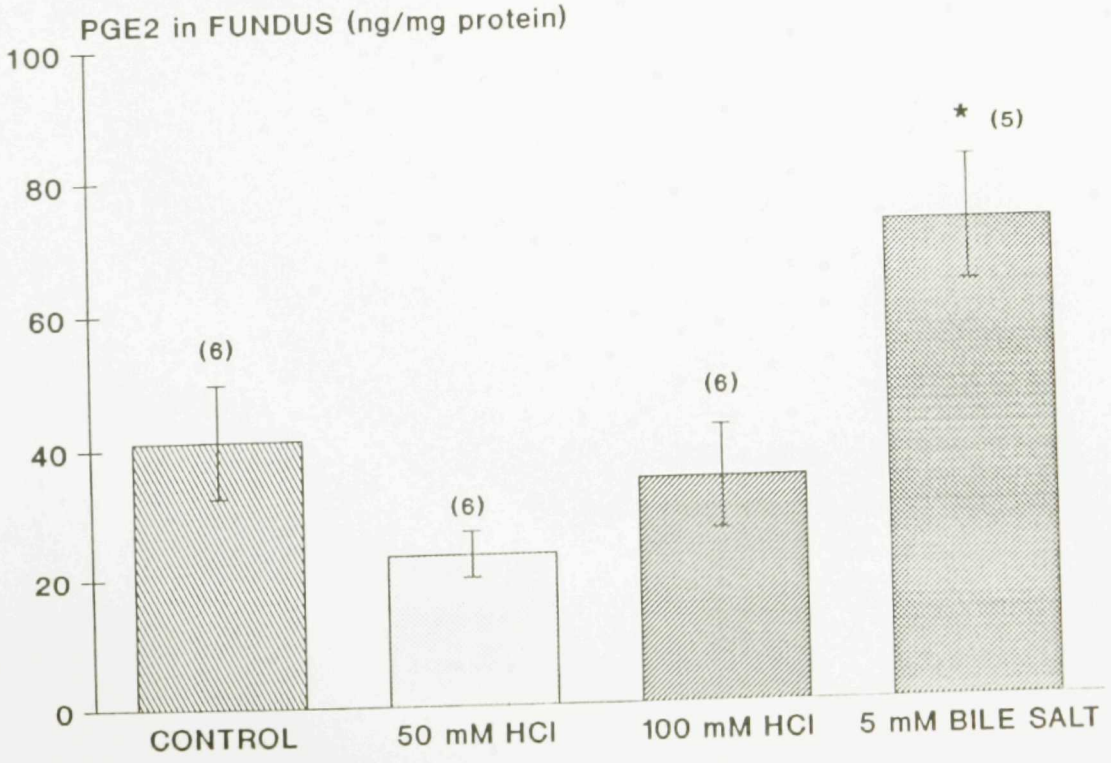
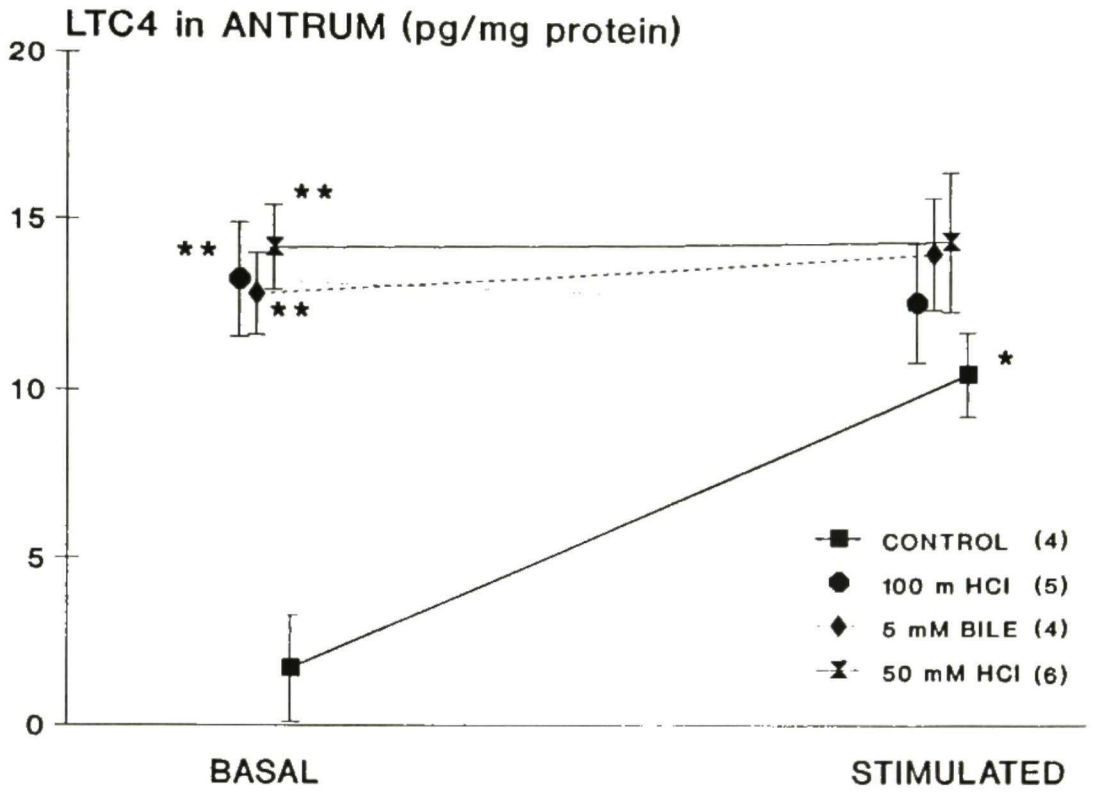


Figure 16. The effect of administration of an intragastric load of either acid (50 or 100 mM HCl) or 5 mM bile salts on basal versus A23187-calcium ionophore stimulated $LTC_4$ generation in the antrum (**Panel A**) and the fundus (**Panel B**). Values are means $\pm$ S.E.M. (#) = number of animals assayed, * $P < 0.05$ and ** $P < 0.01$ versus basal control.

PANEL A




PANEL B

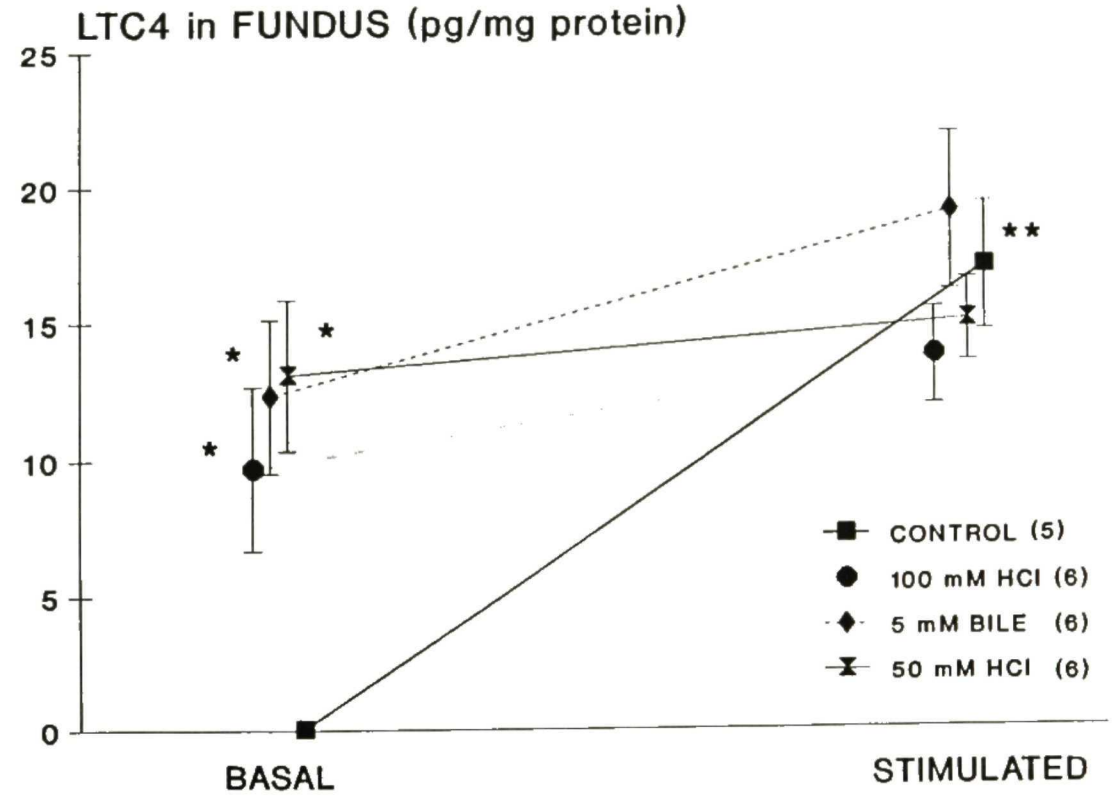
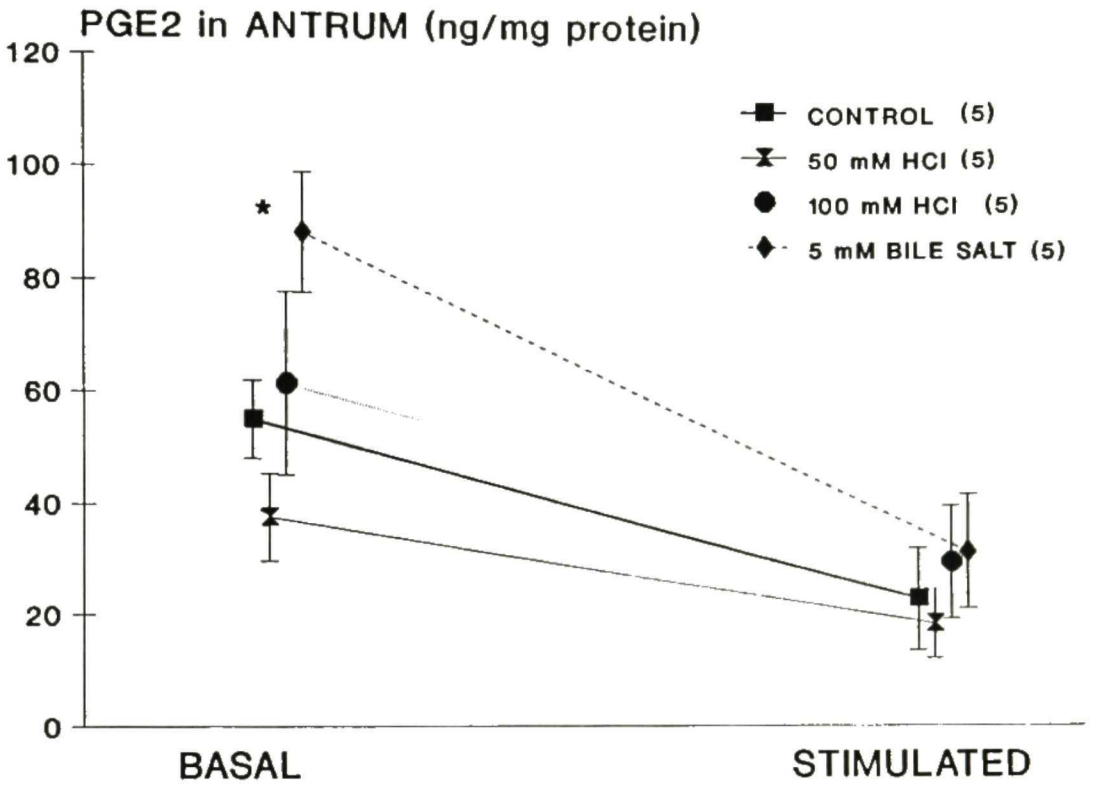
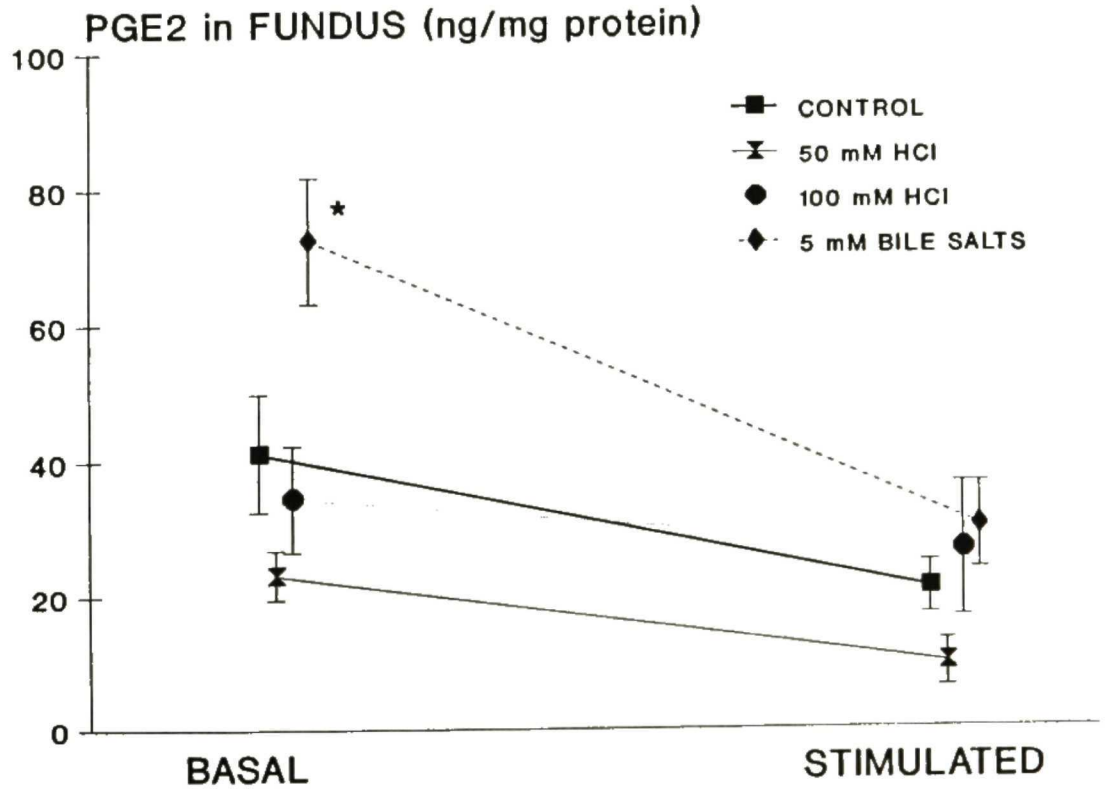


Figure 17. The effect of administration of an intragastric load of either acid (50 or 100 mM HCl) or 5 mM bile salts on basal versus A23187-calcium ionophore stimulated $PGE_2$ generation in the antrum (**Panel A**) and the fundus (**Panel B**). Values are means $\pm$ S.E.M. (#) = number of animals. * $P < 0.05$ versus basal control, $P < 0.05$ versus basal bile and # $P < 0.05$ versus basal control.

PANEL A




PANEL B



**Effect of Inhibition of Sensory Afferent Nerves by Lidocaine on Gastric Emptying and Mucosal Eicosanoid Generation After Exposure of the Gastric Mucosa to Acid or Bile Salts Load**

Studies were performed to determine the role of sensory afferent nerves in the selective induction of eicosanoids in response to noxious stimuli. Sensory input was inhibited by lidocaine (iv bolus, 2.2 mg/kg followed by a continuous iv infusion 66 μg/kg/min).

Neither the 100 mM HCl load nor lidocaine had a significant effect on mean FER (Figure 18, panel A). The 5 mM bile salt load decreased mean FER and lidocaine inhibited this bile salt-induced decrease in mean FER (Figure 19, panel B). Figure 19 illustrates FER during the early (0-10 min) interval following a liquid load. Results for the 100 mM HCl load are in panel A and results for the 5 mM bile salt load are in panel B. When compared to control, lidocaine alone significantly decreased early FER. But, lidocaine given with the acid and bile load altered early FER such that FER was no longer significantly different from control or the acid and bile load.

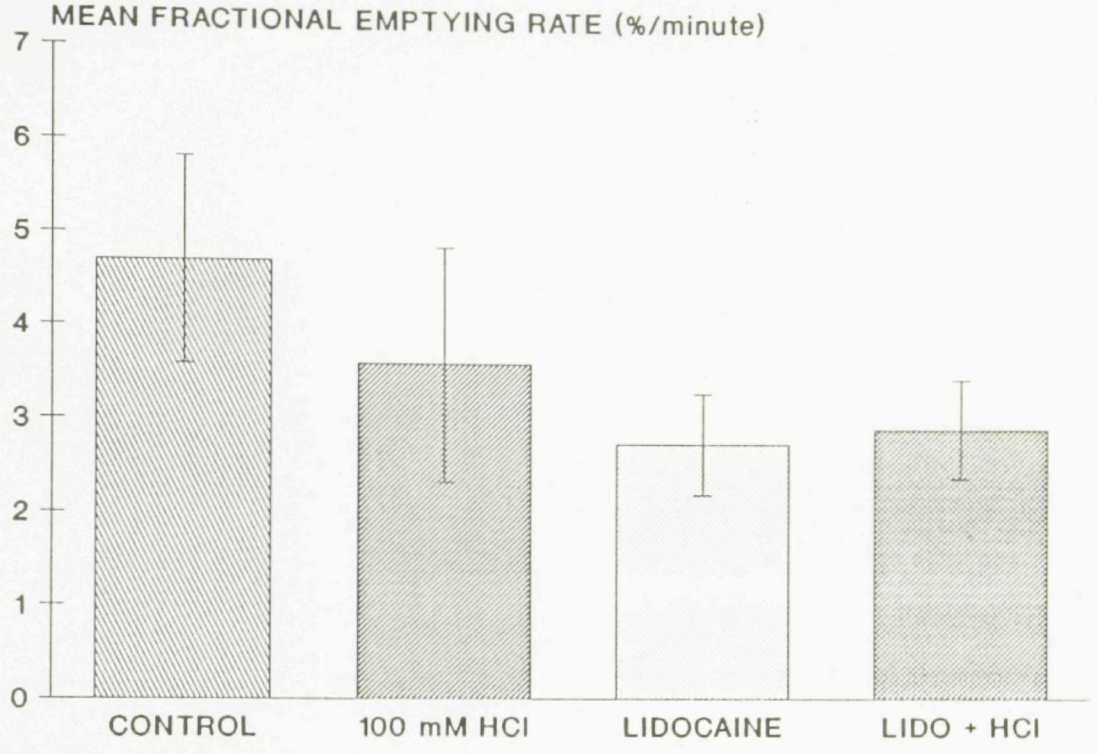
Table VIII summarizes the effect of lidocaine and lidocaine plus the acid or bile salt load on macroscopic damage scores and Table IX summarizes the effect on microscopic damage scores. The intravenous infusion of lidocaine did not produce either macro- or microscopic damage to the gastric mucosa. Lidocaine also had no effect on damage scores following the intragastric administration of the acid or bile salt loads. As in the previous study, due to the rigorous criteria followed for the MMC counts, many biopsies were deferred from the count due to incorrect orientation or missing muscularis mucosa. Thus, no statistical analysis

was performed. Table X summarizes the effect of all the treatments on mucosal mast cell counts. As can be seen from the table, there was no difference in MMC counts. Using a subjective approach, there appeared to be a difference in the intensity of the staining of the mast cells. This difference in staining may be due to a partial degranulation of the mast cells. Figure 20 shows MMC in typical sections of treated gastric mucosal tissue. While the acid load is associated with fewer MMC, because of the small number of samples, statistical analysis could not be performed.

When compared to control, lidocaine alone had no effect on $LTC_4$ generation in the antrum or the fundus. However, lidocaine blocked the stimulation of $LTC_4$ generation in the antrum and fundus in response to exposure to 100 mM HCl (Figure 21) or 5 mM bile salts (figure 22). The IV infusion of lidocaine did not alter mucosal $PGE_2$ generation in the antrum or fundus after exposure to water, 100 mM HCl (Figure 23) or 5 mM bile salts (figure 24).

Figure 18. The effect of lidocaine (2.2 mg/kg IV bolus followed by IV infusion 66 $\mu$g/kg/min) and an intragastric load of 100 mM HCl (Panel A) or 5 mM Bile salts (Panel B) on mean (0-60 minute) fractional emptying rate. Values are means $\pm$ S.E.M. of 5 animals. * $P < 0.05$ versus control, # $P < 0.05$ versus 5 mM bile.

PANEL A




PANEL B

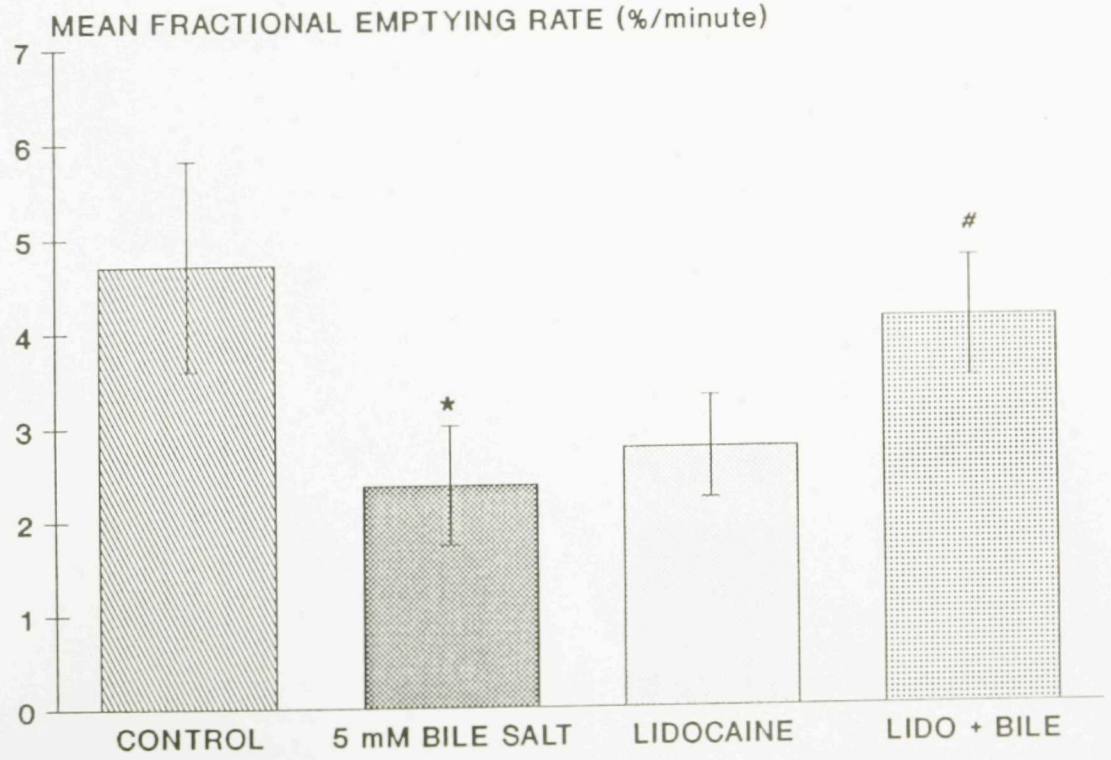
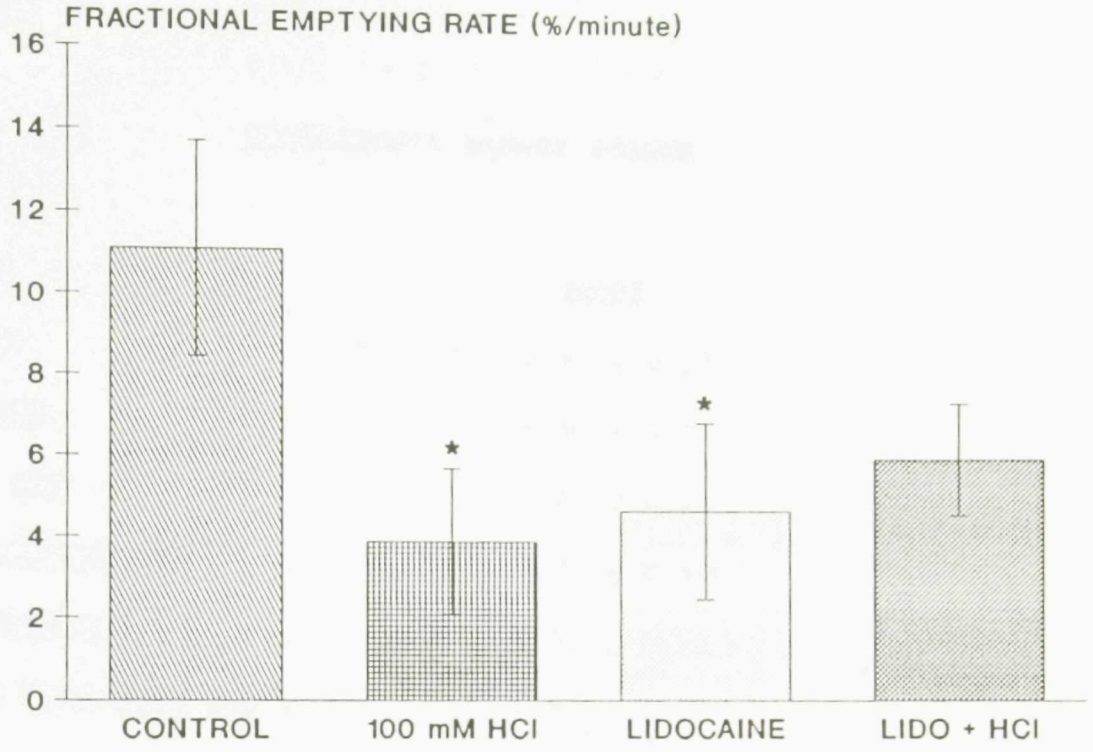


Figure 19. The effect of lidocaine (2.2 mg/kg IV bolus followed by IV infusion 66 $\mu$g/kg/min) and an intragastric load of 100 mM HCl (Panel A) or 5 mM Bile salts (Panel B) on the early (0-10 minute) interval of fractional emptying rate. Values are means $\pm$ S.E.M. of 5 animals, * $P < 0.05$ versus control.

PANEL A




PANEL B

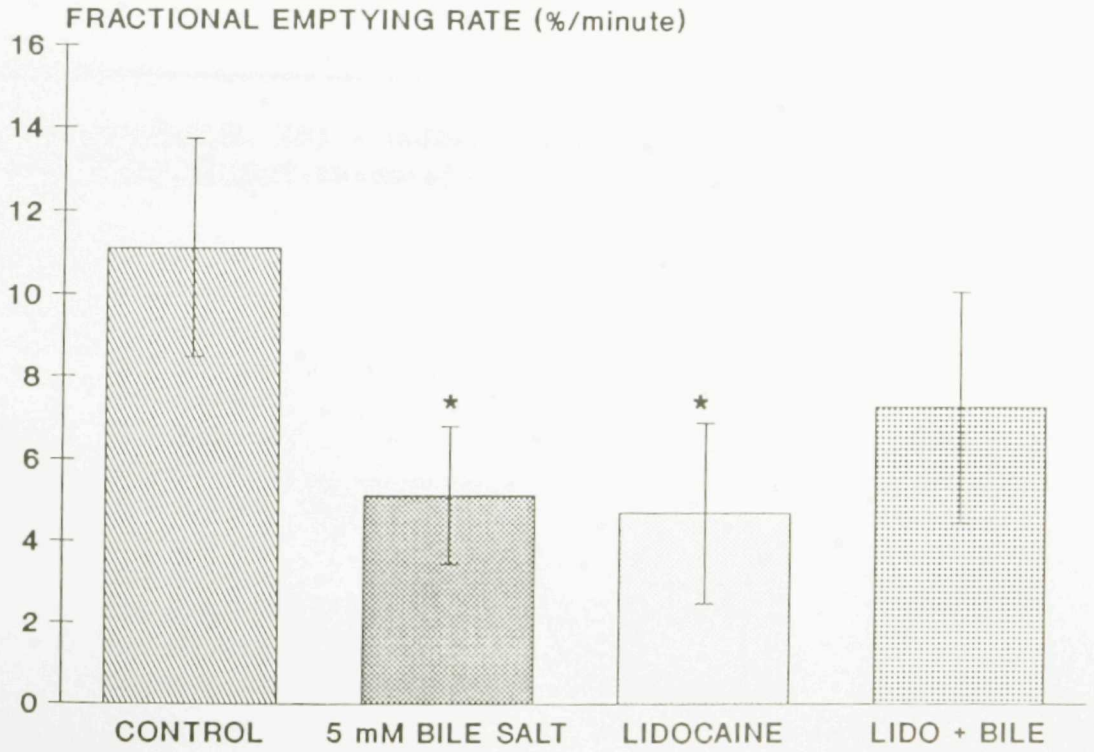


TABLE VIII

MACROSCOPIC DAMAGE SCORES

| TREATMENT | SCORE |
|---|---|
| CONTROL (6) | 0.17 ± 0.17 |
| LIDOCAINE (6) | 0.50 ± 0.23 |
| 100 mM HCl (6) | 0.33 ± 0.21 |
| LIDO + 100 mM HCl (5) | 0.75 ± 0.24 |
| 5 mM BILE SALT (6) | 0.50 ± 0.22 |
| LIDO + 5 mM BILE SALT (5) | 0.80 ± 0.20 |

Values are means ± S.E.M. (#) = number of animals evaluated. Antral and fundal scores were averaged to obtain one overall mean.

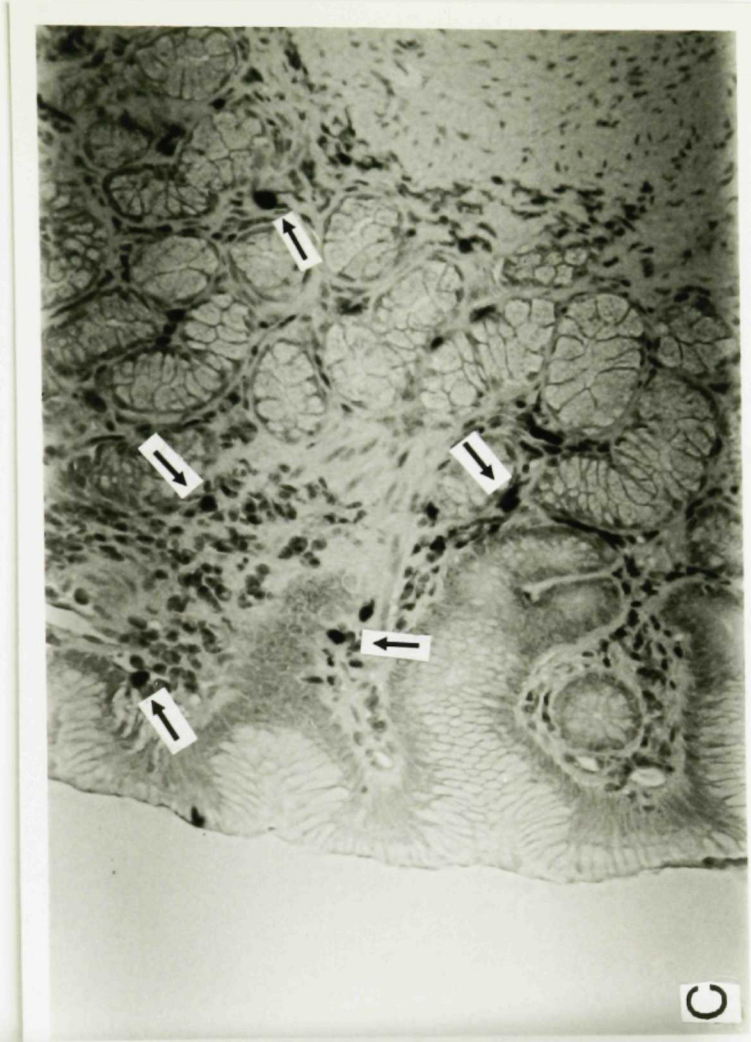
TABLE IX

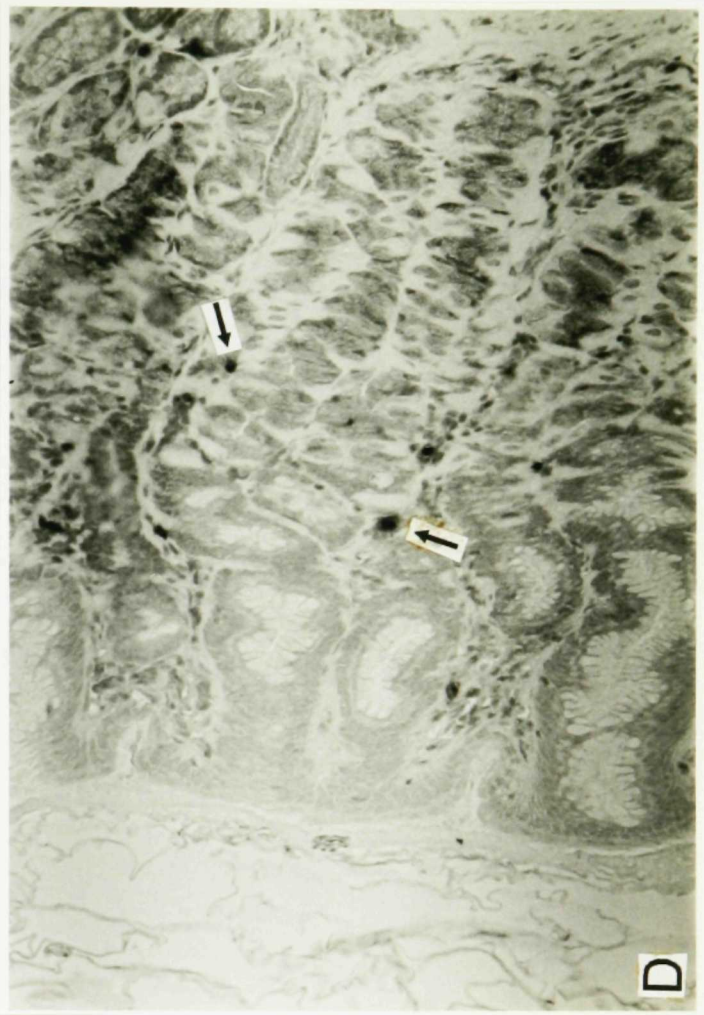
MICROSCOPIC DAMAGE SCORES

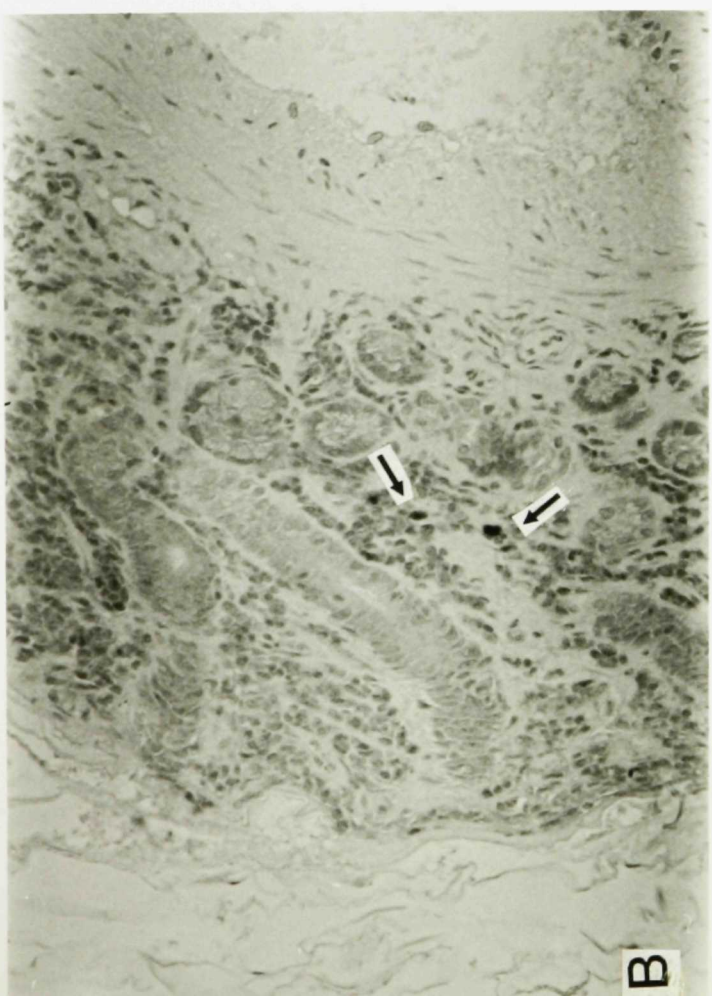
| TREATMENT | SCORE |
|---|---|
| CONTROL (6) | 1.25 ± 0.34 |
| LIDOCAINE (6) | 0.92 ± 0.20 |
| 100 mM HCl (6) | 1.81 ± 0.25 |
| LIDO + HCl (5) | 1.25 ± 0.45 |
| 5 mM BILE SALTS (6) | 1.50 ± 0.38 |
| LIDO + 5 mM BILE SALTS (5) | 1.17 ± 0.25 |

Values are the means ± S.E.M. (#) = number of animals assayed. Thus, antral and fundal scores were averaged to obtain one overall mean.

Figure 20. Photomicrographs illustrating toluidine blue staining of mucosal mast cells (shown by arrows) in rhesus monkey gastric mucosa. Mast cells are seen as dark cells against a pale background stain. Magnification is 226x. **Panel A** is water load, **Panel B** is 100 mM HCl load, **Panel C** is water load and IV lidocaine (2.2 mg/kg bolus, 66$\mu$g/kg/min infusion) **Panel D** is 100 mM HCl load and IV lidocaine.


C


D


B

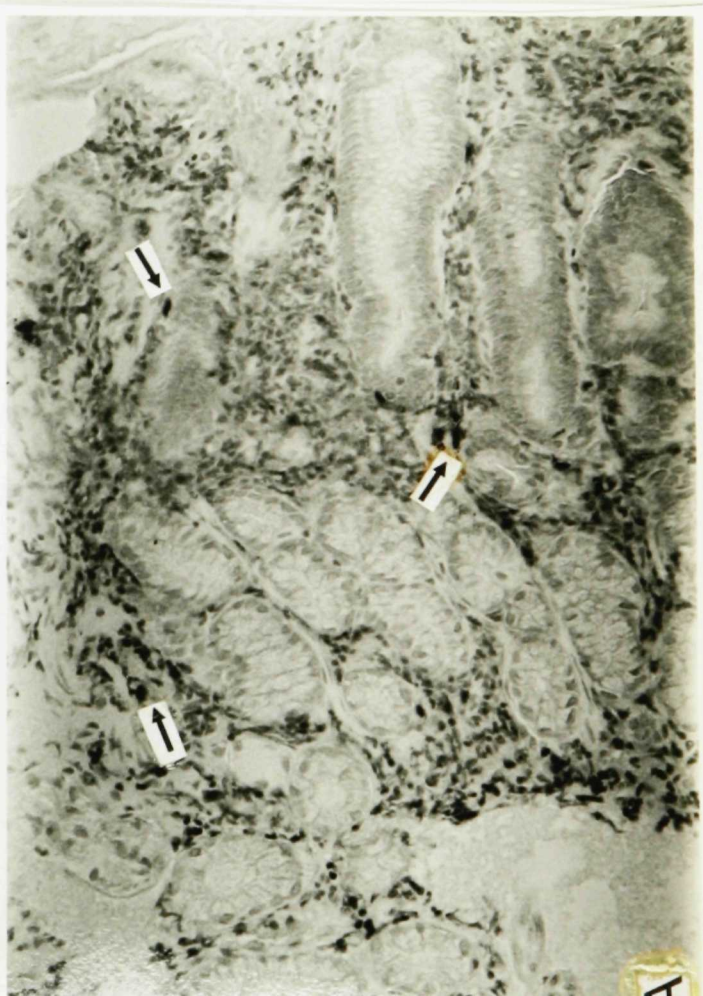

A

TABLE X

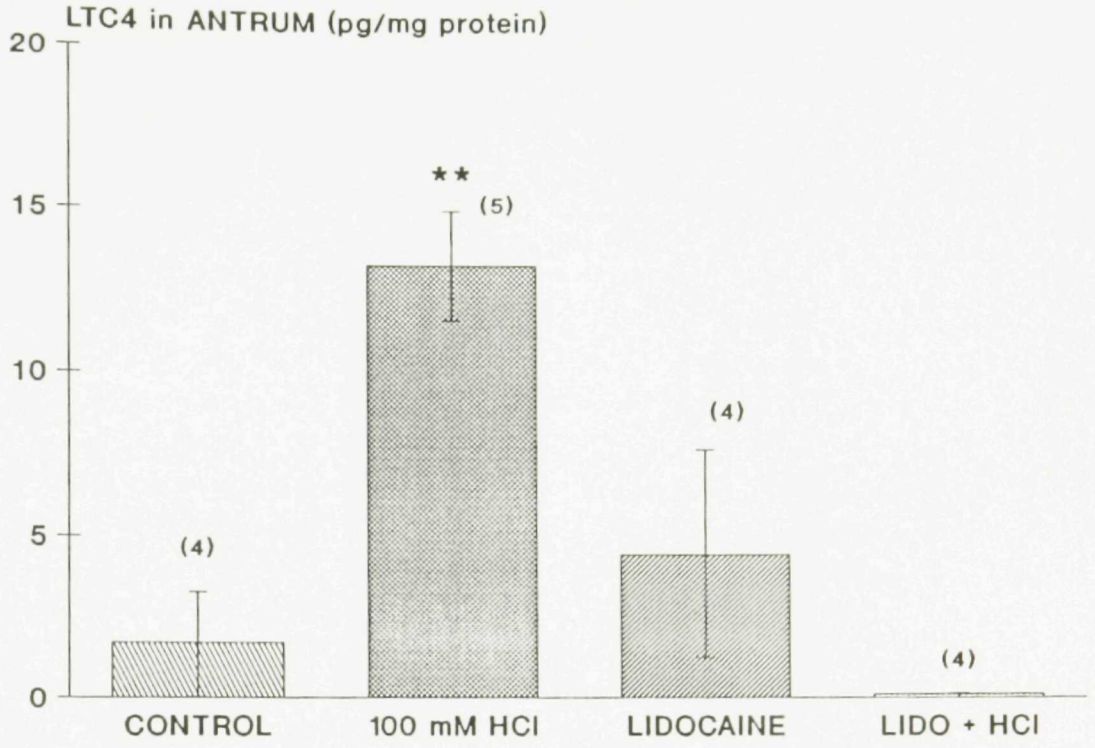
MUCOSAL MAST CELL COUNTS

| TREATMENT | MMC/0.46 $mm^2$ |
|---|---|
| CONTROL (3) | 5.6 ± 2.3 |
| 100 mM HCl (3) | 5.3 ± 1.8 |
| 5 mM BILE SALT (1) | 1.0 ± 1.0 |
| LIDOCAINE (5) | 6.5 ± 2.2 |
| LIDO + 100 mM HCl (1) | 5.2 ± 5.2 |

(#)= number of animals assayed.

Figure 21. The effect of lidocaine (2.2 mg/kg IV bolus followed by IV infusion 66 $\mu$g/kg/min) and an intragastric load of 100 mM HCl on $LTC_4$ generation in the antrum (**Panel A**) or the fundus (**Panel B**). Values are means $\pm$ S.E.M., * $P < 0.05$ versus control, (#) = number of animals.

PANEL A




PANEL B

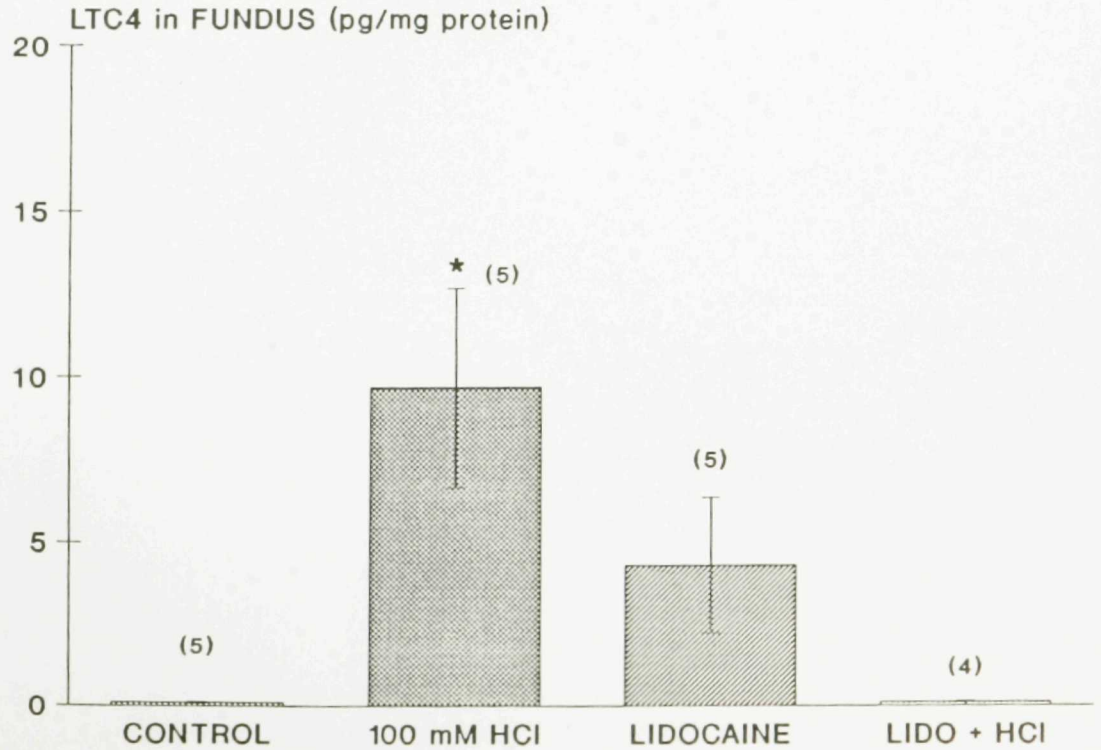
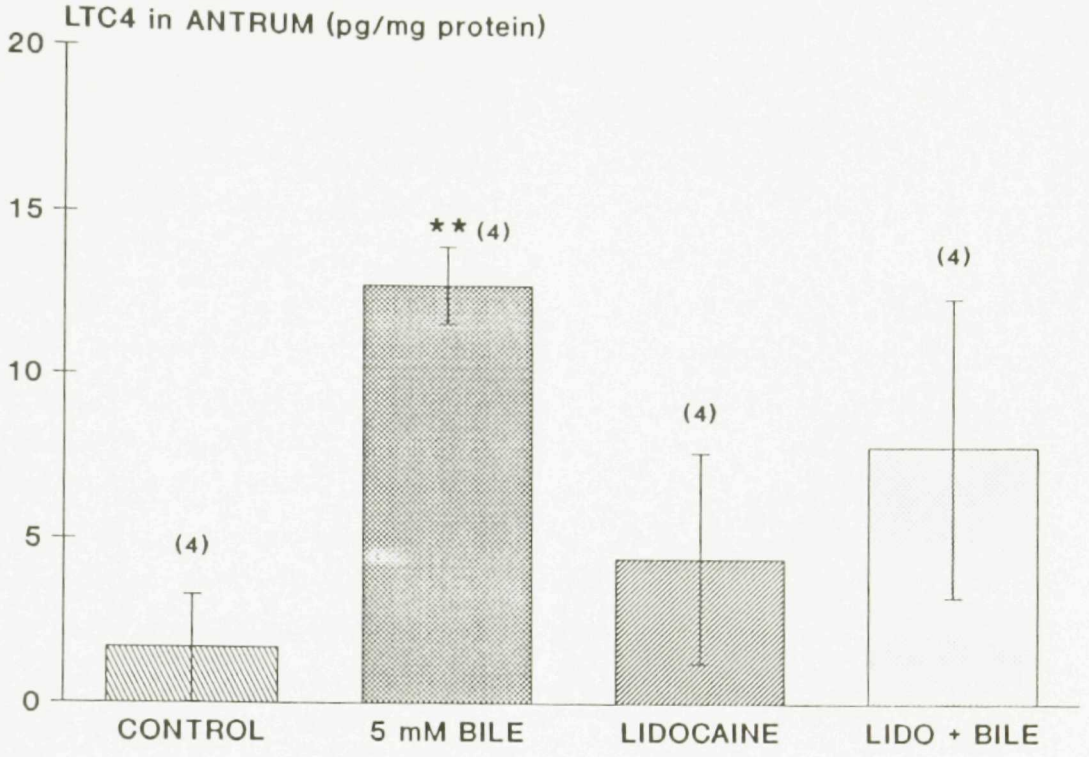


Figure 22. The effect of lidocaine (2.2 mg/kg IV bolus followed by IV infusion 66 $\mu$g/kg/min) and an intragastric load of 5 mM bile salts on $LTC_4$ generation in the antrum (**Panel A**) or the fundus (**Panel B**). Values are means $\pm$ S.E.M., * $P < 0.05$ versus control, (#) = number of animals.

PANEL A




PANEL B

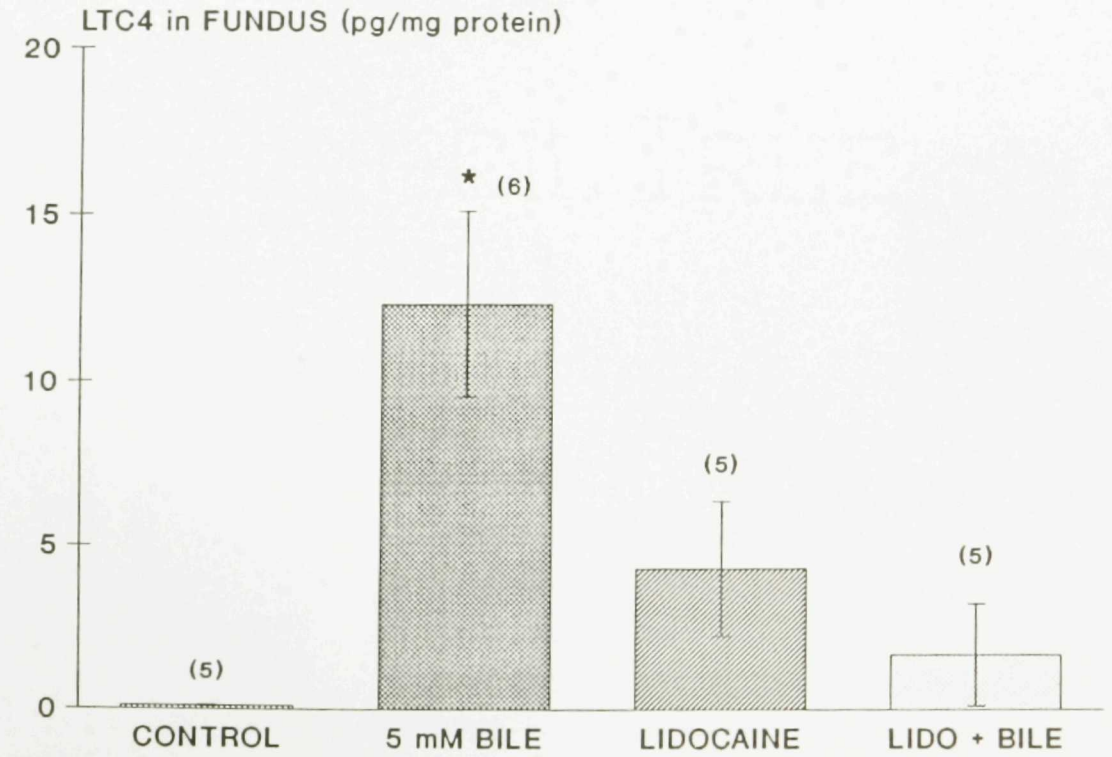
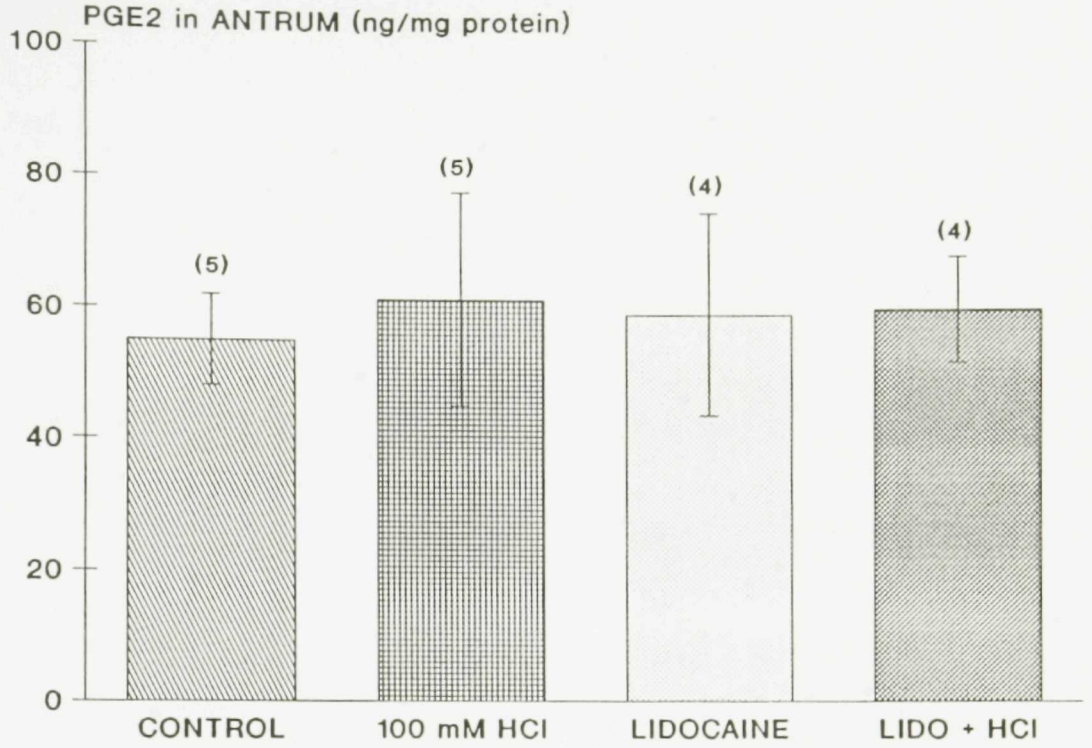


Figure 23. The effect of lidocaine (2.2 mg/kg IV bolus followed by IV infusion 66 $\mu$g/kg/min) and an intragastric load of 100 mM HCl on $PGE_2$ generation in the antrum (**Panel A**) or the fundus (**Panel B**). Values are means $\pm$ S.E.M. (#) = number of animals.

PANEL A




PANEL B

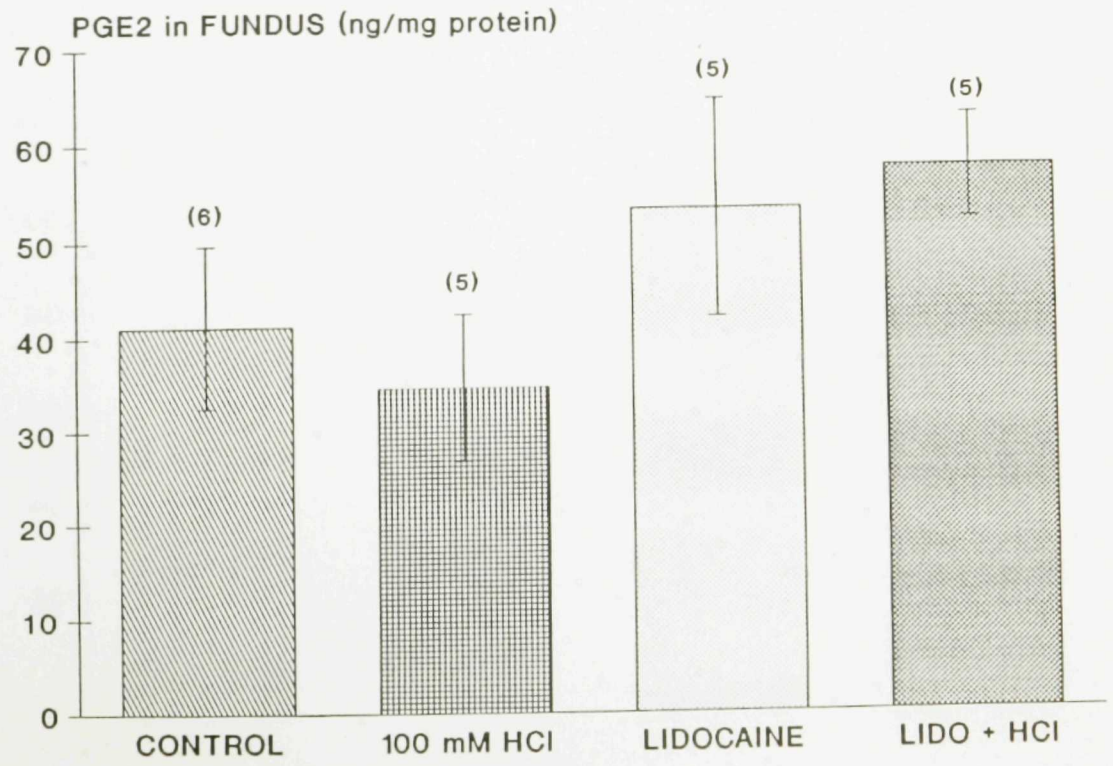
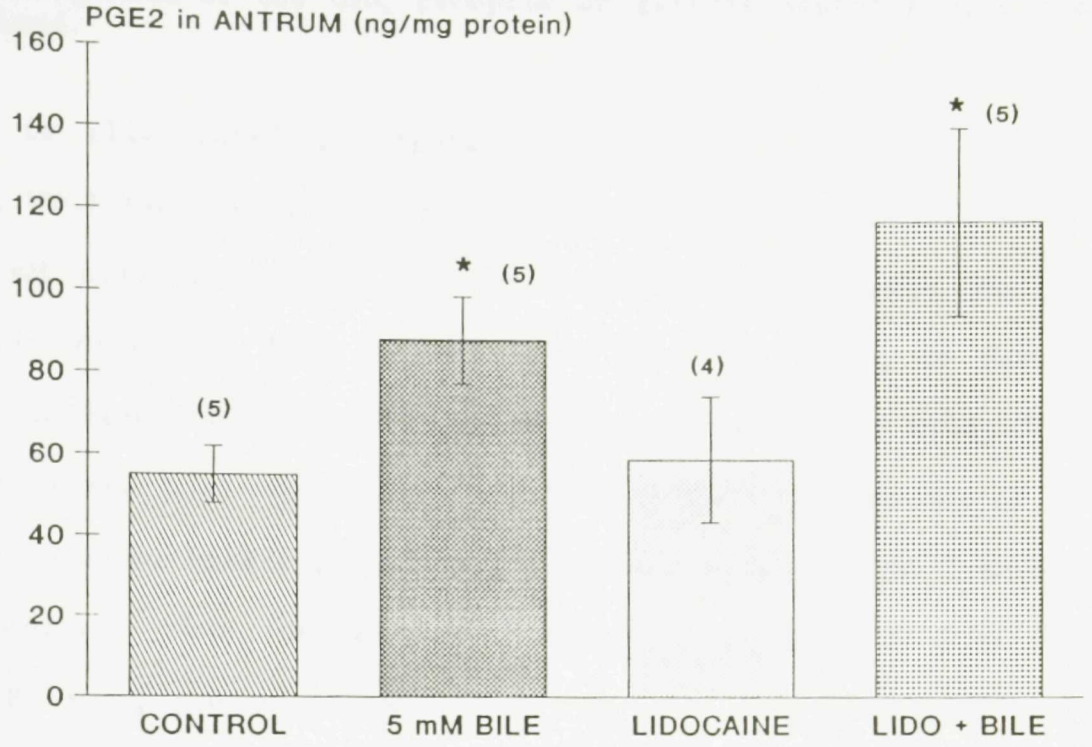
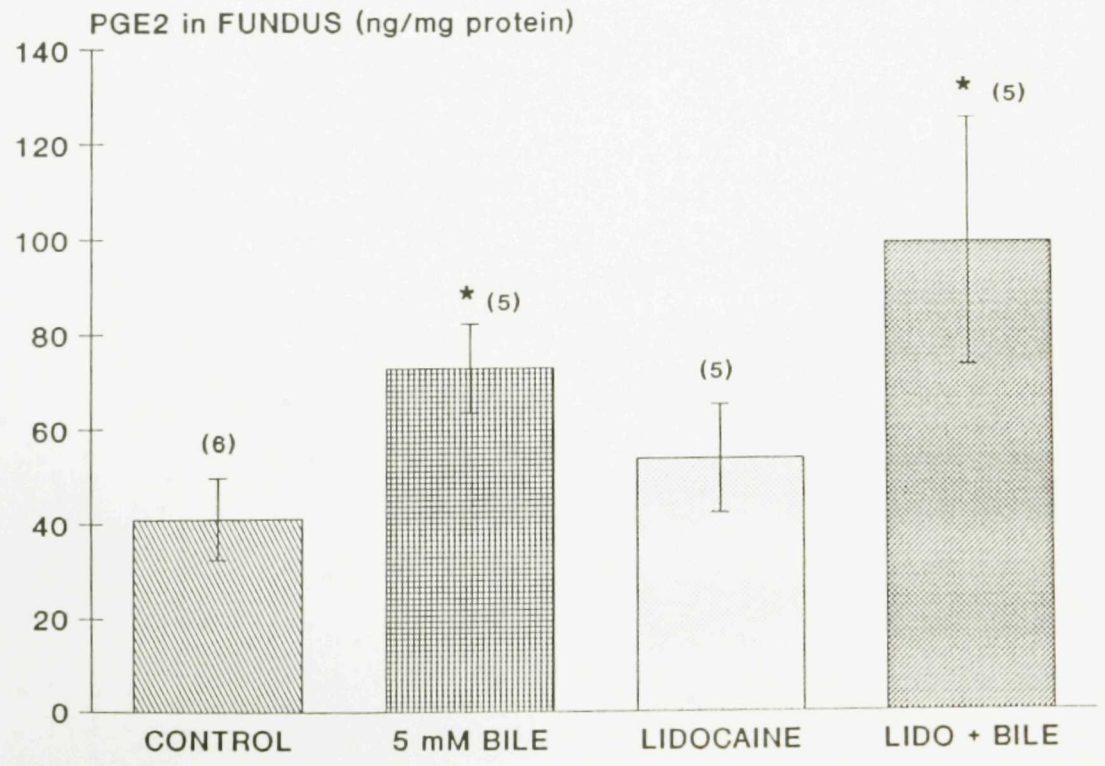


Figure 24. The effect of lidocaine (2.2 mg/kg IV bolus followed by IV infusion 66 $\mu$g/kg/min) and an intragastric load of 5 mM bile salts on $PGE_2$ generation in the antrum (**Panel A**) or the fundus (**Panel B**). Values are means $\pm$ S.E.M., * $P < 0.05$ versus control, (#) = number of animals.

PANEL A




PANEL B

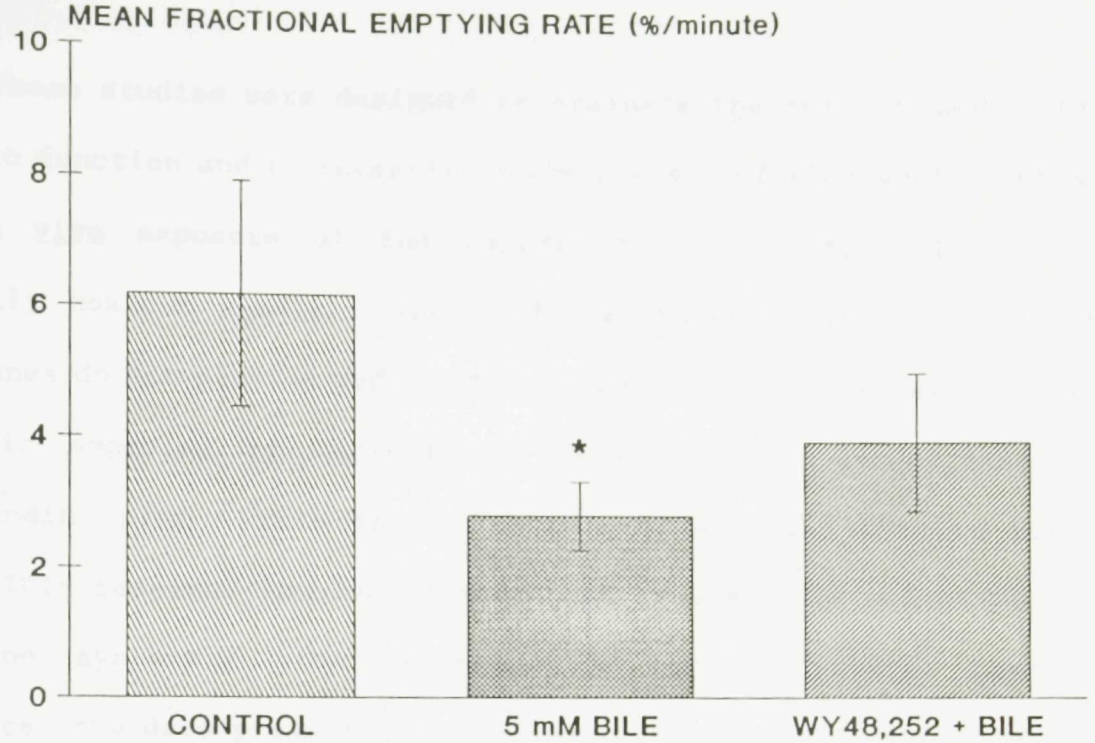


**Effect of antagonism of the $LTD_4$ receptor on gastric emptying of a 5 mM bile salt load.**

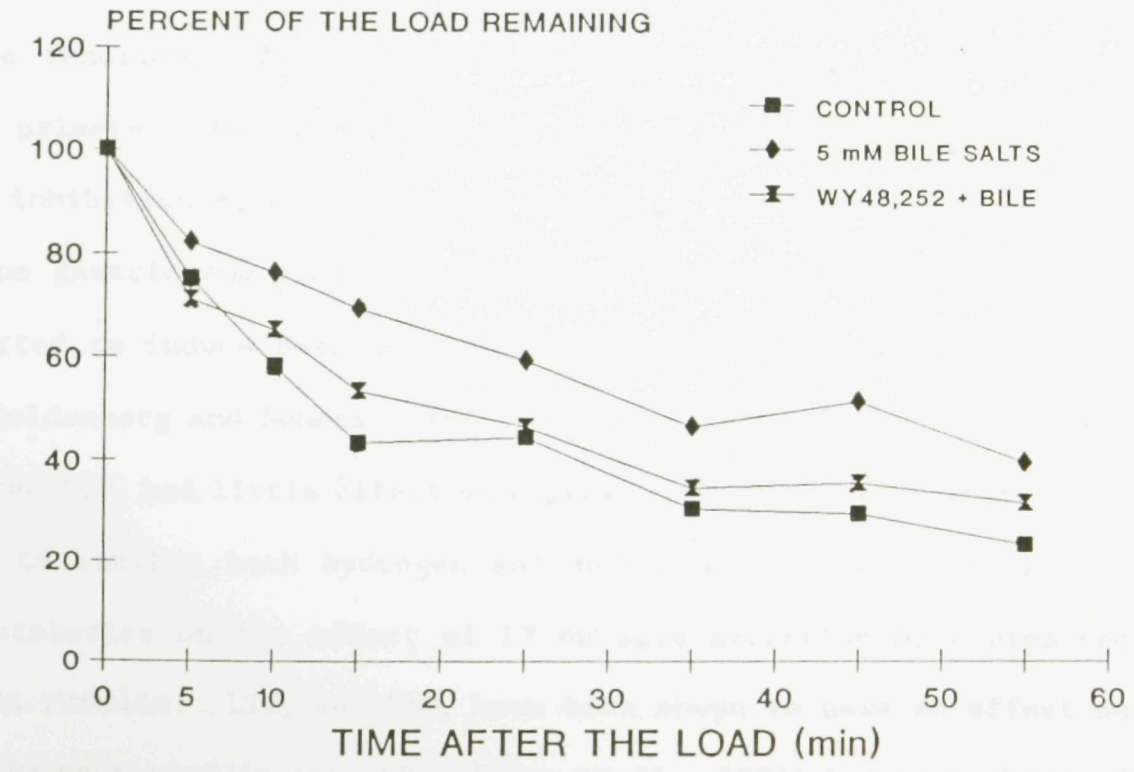
As is illustrated in figure 25 (panel A), the $LTD_4$ receptor antagonist, WY48,252, tended to suppress the decrease in mean FER induced by the 5 mM bile salt load such that WY48,252 plus bile was not significantly different from control or 5 mM bile. This is further illustrated in figure 25 (panel B), which shows the effect of WY48,252 on the percent of the load remaining. WY48,252 shifted the percent of the load remaining curve toward the control curve and away from the 5 mM bile salt load curve. Thus, antagonism of the $LTD_4$ receptor by WY48,252, returned FER toward control values.

Figure 25. The effect of WY48,252 (2.5 mg/kg orally), an $LTD_4$ receptor blocker, on fractional emptying rate following a 5 mM bile salt load. Values are means $\pm$ S.E.M. of 5 animals. **Panel A.** Illustrates mean fractional emptying rate measured over the 60 minute postload period following a 5 mM bile salt load. **Panel B.** The effect of WY48,252 (2.5 mg/kg orally) on percent of the 5 mM bile salt load remaining. Values are means of 5 animals.

PANEL A




PANEL B



# DISCUSSION

These studies were designed to evaluate the role of leukotrienes in gastric function and to investigate the pattern of eicosanoid synthesis after *in vivo* exposure of the gastric mucosa to physiological but potentially noxious agents. The findings presented here suggest that leukotrienes do not play an important role in the physiological regulation of gastric emptying or secretion. However, both leukotrienes and prostaglandins play a role in the gastric mucosal response to noxious agents. This response involves a selective induction of gastric mucosal leukotriene synthesis after exposure to noxious luminal contents. Furthermore, the data presented here suggest that the selective induction of leukotrienes is mediated via a neural reflex.

Recent studies have begun to elucidate the effect of leukotrienes on gastric function. The present investigation is the first to use a conscious primate model to examine the role of exogenously administered $LTD_4$, the inhibition of endogenous LT synthesis, or blockade of the $LTD_4$ receptor on gastric emptying and secretion. Although leukotrienes have been reported to induce dose-dependent contractile responses in the rat stomach (Goldenberg and Subers, 1982), our studies found that exogenously administered $LTD_4$ had little effect on gastric emptying. In contrast, $LTD_4$ was found to inhibit both hydrogen and sodium ion output. Studies in other laboratories on the effect of LT on acid secretion have produced conflicting results. $LTC_4$ and $LTD_4$ have been shown to have no effect on gastric acid secretion in cats (Pendleton et al., 1986) but were observed to stimulate acid secretion from isolated rabbit gastric parietal cells

(Magous et al., 1983). The present results, support the more recent studies of Pawlik et al. (1987) in which an intra-arterial infusion of $LTC_4$ or $LTD_4$ in dogs produced a dose-dependent suppression of acid secretion.

The mechanism by which $LTD_4$ inhibits acid and sodium secretion in the primate stomach is unclear. $LTD_4$ may act directly on parietal cells since $LTC_4$, the precursor of $LTD_4$, has been shown to inhibit acid formation in isolated gastric glands (Konturek et al., 1987) or indirectly, by suppressing gastric mucosal blood flow (Pawlik et al., 1987). The first hypothesis is supported by decreased hydrogen and sodium ion secretion in the face of the absence of changes in either blood pressure or heart rate in the present study. However, the vasoconstrictor effect of the LT cannot be overlooked entirely; the mesenteric vascular bed is very sensitive to LT (Eimerl et al., 1986) and thus regional hemodynamic changes could occur without systemic changes.

The present study shows that $LTD_4$ alone is not damaging to the gastric mucosa and supports studies by Pihan et al. (1988), who showed that infusion of $LTC_4$ or $D_4$ in rats did not cause extensive mucosal necrosis or hemorrhagic erosions. Other studies have demonstrated that although LT themselves do not appear to produce gastric ulceration per se, they can augment the extent of gastric injury induced in the rat by agents such as ethanol and acid (Wallace and MacNaughton, 1988; Konturek et al., 1988).

The data obtained using the 5-lipoxygenase inhibitor, NDGA, and the $LTD_4$ receptor antagonist, WY48,252, further supports the theory that leukotrienes do not play a major role in the physiological regulation of gastric function. Neither NDGA nor WY48,252 altered mean fractional

emptying rate or gastric ion secretion. Although high doses of WY48,252 decreased early FER, it is thought that this effect is due to a side effect of WY48,252, perhaps its acting to inhibit CO or as a partial agonist (Chang et al., 1988). Thus, these studies are consistent with previous studies (Pawlik et al., 1987; Konturek et al., 1987), suggesting that under physiological conditions, LT do not play a role in the regulation of gastric function.

In summary, these data suggest that under physiological conditions, endogenous $LTD_4$ does not play a role in the gastric emptying of a liquid load in primates. The effects on hydrogen and sodium ion secretion may be due to a direct action of $LTD_4$ on the parietal cell or indirectly via a decrease in mucosal blood flow. However, this does not preclude a role for the LT in the gastric mucosa's response to potentially noxious stimuli.

The results presented here are the first to investigate the generation of gastric mucosal eicosanoids after exposure of the gastric mucosa to physiological but potentially damaging agents, and thus provides a sensitive and relevant model to study the gastric mucosal barrier. The present results demonstrate that a mild distention stimulus does not increase the generation of $LTC_4$. However, the gastric mucosa releases $LTC_4$ in response to physiological levels of acid and releases both $LTC_4$ and $PGE_2$ in response to bile salts. Two PG analogs, Risoprostil, a $PGE_1$ analog, and 16, 16-dimethyl $PGE_2$ have been shown to decrease $LTC_4$ generation and to attenuate damage caused by ethanol (Wallace et al. 1988; Boughton-Smith and Whittle, 1988). In this study however, $PGE_2$ generated by the 5 mM bile salt load did not alter $LTC_4$ generation. This suggests that under

physiological conditions these two products of AA oxidation are independently regulated. In addition, the selective induction of eicosanoid synthesis suggests that the cellular source for the generation of LT and PG is different. Recent studies in other laboratories have shown that LT are released in response to 40% and above concentrations of ethanol (Peskar et al., 1986) or acid (6N) which produce damage to the gastric mucosa (Osada et al., 1990). However, the release of $LTC_4$ in the present study is not correlated with gastric mucosal damage, which is in agreement with the report of Wallace et al. (1988) showing the stimulation of gastric $LTC_4$ synthesis by ethanol is independent of the production of hemorrhagic damage. Taken together, these data support the hypothesis that the LTs are released in response to noxious agents and contribute to the persistence of gastric lesions, but are not involved in the initial onset of mucosal damage (Osada et al., 1990).

The antrum and the fundus have different structural and functional characteristics. The antrum receives more gastric mucosal blood flow than the fundus and the vasculature is arranged such that the blood delivers bicarbonate to the surface epithelial cells and carries away hydrogen ion that has back-diffused. The fundus serves as a reservoir for food before it is emptied into the duodenum, while the antrum functions as a sieve. Thus, it likely that the eicosanoid generation profile is different in the antrum and fundus. In the present study, there was no significant difference between $LTC_4$ or $PGE_2$ levels in the antrum when compared to the fundus. Since the fundus serves as a reservoir for food, the mucosa of the fundus is exposed to the load for a longer period of time than is the

antrum. In this study, however, eicosanoid generation was not stimulated by the longer exposure time to the luminal contents.

Previous investigations have suggested that LT can be released from MMC and that PG can be released from SEC. Traditionally mast cells and their products have been thought to be involved in allergic or hypersensitivity states, but they have also been implicated in a variety of non-immunologic functions (Shanahan et al., 1985). Unchecked mediator release from tissue mast cells has considerable potential for tissue destruction. Thus, mast cells, like other secretory cells, must be subject to precise regulation. The mast cell data provides evidence that MMC are present in the rhesus monkey gastric mucosa. Although no statistically significant data regarding mast cell number were obtained in the present study using the criteria of Strobel et al. (1981), the variable density of MMC staining suggests that MMC can degranulate in response to luminal contents, leading to a diminution of mast cell granularity. Recent investigations have focused on the role of the mast cell in the pathogenesis of acute gastric ulceration. There have been several reports that stimulation of mast cell degranulation by reserpine or polymyxin B results in severe gastric hemorrhage and necrosis (Ogle and Lau, 1980; Cho and Ogle 1979). Further evidence that mast cells are involved in acute gastric ulceration was provided by Galli et al. (1985), who reported that genetically mast cell deficient mice were less susceptible to ethanol-induced gastric damage than mice with normal mast cell populations. Moreover, agents reported to be mast cell stabilizers have been shown to inhibit gastric damage and the changes in acid secretion and motility induced by reserpine, aspirin and ethanol (Ogle and

Lau, 1980; Canfield and Curwain, 1983; Takeuchi et al., 1984; Wallace et al., 1988). Since oral administration of ethanol has been shown to increase release of $LTC_4$ and since mast cells are likely to be one of the primary cellular sources of $LTC_4$, mast cell stabilizers might be expected to reduce ethanol-induced $LTC_4$ release. In a study by Beck et al. (1989), pretreatment of rats with the mast cell stabilizer, FPL-52694, produced a significant reduction of gastric $LTC_4$ synthesis, although this effect did not appear to be important in terms of protection afforded to the gastric mucosa.

One possible mechanism for the selective induction of LT synthesis from MMC is a neural pathway. Since there are many nerves in the gastric mucosa, luminal contents could stimulate the nerves which in turn stimulate MMC and cause the release of LT. The present studies using lidocaine, a local anesthetic, to block sensory afferent input suggest that a local neural pathway may be responsible for the selective generation of $LTC_4$. Lidocaine acts to stabilize the ionic fluxes of the neuronal cell membrane inhibiting the generation of a nervous impulse. When a local anesthetic, such as lidocaine, is applied to a nerve fiber, the threshold for excitation increases, impulse conduction slows, and the ability to generate an action potential is abolished. All of these effects are due to the binding of the local anesthetic to sodium channels, which results in the blockade of the sodium channel (Hondeghem and Miller, 1987). These data suggest that lidocaine inhibited the generation of a nervous impulse in sensory afferents and secondarily inhibited the release of LT from MMC. There is evidence that neuro-immune interactions may be important as pathogenetic mechanisms in humans. In a recent study, it was

postulated that the silencing of hyperactive enteric nerves in patients with ulcerative colitis by a local anesthetic agent may influence the inflammatory response. A topical treatment with a lidocaine (2 %) gel in these subjects resulted in restored mucosal integrity and the disappearance of extravasal lymphocytes (Bjork et al., 1991).

Besides inhibiting ionic fluxes in neural tissue, local anesthetics have been documented to have membrane stabilizing properties. They have been found to increase the resistance of erythrocytes to hypotonic hemolysis, presumably through membrane expansion induced by the drug (Roth and Seeman, 1972). In addition, local anesthetics inhibit mitochondrial swelling by phospholipase A and also to inhibit the transport of potassium and calcium across the mitochondrial membrane, possibly by membrane stabilization (Seeman, 1972). Thus, lidocaine has been shown to protect cardiac cell structures during ischemia by stabilization of mitochondria membranes (Schaub et al., 1977). In contrast to cardiac tissue, gastric tissue has fewer mitochondria and is less dependent on them for metabolism than cardiac tissue. In addition, lidocaine has been shown to have differential effects on normal and infarcted cardiac tissue (Kupersmith et al., 1975). Specifically, lidocaine has very few electrophysiological effects on normal cardiac tissue. Thus, while the membrane stabilizing effect of lidocaine cannot be excluded in this study, it does not seem probable that lidocaine's membrane stabilization properties play a role in the results presented here.

Besides providing cardiac protection, lidocaine has also been shown to provide gastric cytoprotection during acute gastric distention in the dog (Pfeiffer, 1989). The gastroprotective effect of lidocaine was

attributed to lidocaine's ability to stabilize cellular and organelle membranes. However, two other membrane stabilizers, zinc sulfate and prednisolone, did not protect against damage induced by acute gastric distention. A neural pathway was not considered in these studies.

In the study presented here, lidocaine inhibited the generation of $LTC_4$ induced by the acid or bile loads, but had no effect on $PGE_2$ generation in response to the bile load. These data support a neural mechanism for the selective induction of $LTC_4$. A neural mechanism of selective induction of $LTC_4$ is further supported by the gastric emptying data. The 5 mM bile salt load decreased mean FER and the intravenous infusion of lidocaine was found to return the mean FER toward control values. In addition, early FER was found to be decreased by the 100 mM HCl and the 5 mM bile salt load, an effect which was partially blocked by lidocaine. The early gastric emptying of a distention stimulus has been traditionally ascribed to the vagal innervation. In the data presented here, lidocaine decreased early (0-10 min) FER after a water load, further supporting the hypothesis that in this model, lidocaine is acting as a neural anesthetic and not as a membrane stabilizer. The study using the $LTD_4$ receptor antagonist, WY48,252, also supports a neural mechanism. WY48,252 tended to suppress the decrease in mean FER induced by the 5 mM bile salt load. Lidocaine, which prevented the bile salt-induced decrease in mean FER, also caused a significant inhibition of $LTC_4$ generation.

The results of the study presented here demonstrate that $LTC_4$ and $PGE_2$ are released in response to physiological concentrations of acid and bile salts. Since the levels of eicosanoids generated did not cause damage to the gastric mucosa, the role of eicosanoids may be in signaling

the presence of luminal contents or in amplifying, but not initiating, the process of ulceration. Thus, in the study presented here, the luminal contents did not cause gastric damage. However, if generated in great enough concentrations, there are several mechanisms through which inflammatory mediators such as LT may play a role in gastric mucosal damage. These mechanisms could be broadly classified as: 1) vascular actions, 2) chemotactic actions, 3) effects on smooth muscle, 4) effects on cell turnover and 5) stimulation of the release of other mediators. Specifically, LT may increase the susceptibility of the mucosa to injury by reducing mucosal blood flow. As a result of a reduced blood flow, the supply of oxygen and nutrients to the mucosa is decreased and a poorly perfused mucosa is less able to remove and buffer back-diffusing acid. LT could also increase vascular permeability which would lead to leakage of plasma protein into the interstitium (Wallace, 1990).

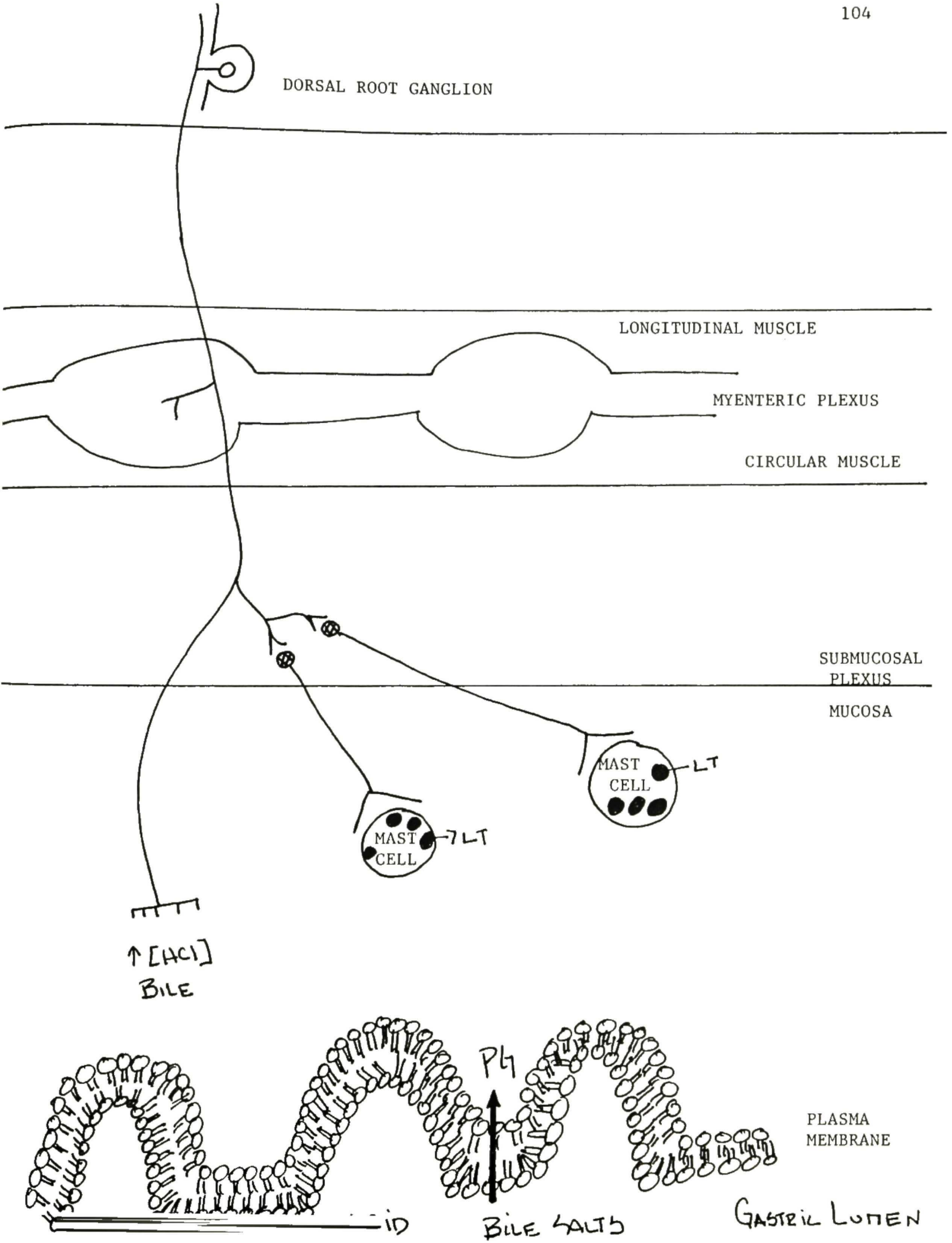
Gastric damage is, like any other kind of wound, accompanied by inflammation. Inflammation can be viewed as a "double-edged sword" since it has two opposing effects. The defensive response acts to prevent the entry of bacteria and the removal of damaged tissue from the site of injury so that repair can take place. The harmful response acts to promote tissue necrosis via the activation of granulocytes and the release of proteolytic enzymes or the reduction of blood flow which decreases the supply of oxygen and nutrients to the tissue.

Most recent research has in the field of gastric mucosal defense has concentrated on characterizing the ability of various mediators to either increase or decrease the susceptibility of the gastric mucosa to acute injury. This has resulted in a list of "good guys" and "bad guys". While

recent research has suggested that the LT are "bad guys", in the study presented here, LT did not cause significant damage to the gastric mucosa. This result suggests that LT may play a role in the detection of luminal contents and the signaling of noxious contents. Thus, LT could act as a local signal which may behave like a call to the enteric nervous system and activate a specific program of motor behavior.

In conclusion, local neuronal processes within the gastric mucosa have been proposed to modulate its integrity and ability to withstand noxious challenge. While it is known that luminal contents can directly damage the gastric mucosa, the role of sensory afferents in the production of damage needs to be investigated further. The results of the present study suggest that there may be a local neural process which is involved in the selective induction of $LTC_4$ generation. This concept is illustrated in figure 26. Luminal contents such as 100 mM HCl or 5 mM bile salts may stimulate a sensory afferent nerve, antidromically stimulating mucosal mast cells to release $LTC_4$ which could then alter mucosal blood flow and/or secretion. In contrast, $PGE_2$ is generated in response to 5 mM bile salts, but not 50 or 100 mM HCl. It is probable that the bile salt load also has a detergent effect on the plasma membrane which causes the generation of $PGE_2$. The results of the studies presented here suggest that mucosal nerves may play a pathogenic role in the inflammatory process in patients with gastric ulcers or ulcerative colitis.

Figure 26. Schematic diagram showing reflex pathway between afferent sensory nerves and mast cells. Modified from Mayer et al., 1988.


DORSAL ROOT GANGLION
LONGITUDINAL MUSCLE
MYENTERIC PLEXUS
CIRCULAR MUSCLE
SUBMUCOSAL PLEXUS
MUCOSA
MAST CELL
LT
MAST CELL
LT
↑ [HCl]
BILE
PLASMA MEMBRANE
BILE SALTS
GASTRIC LUMEN

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